DIFFERENCES IN THE PHARMACOKINETICS OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR AGONISTS IN GENETICALLY OBESE ZUCKER AND SPRAGUE-DAWLEY RATS: IMPLICATIONS OF DECREASED GLUCURONIDATION IN OBESE ZUCKER RATS

Mi-Sook Kim, Sui Wang, Zhongzhou Shen, Christopher J. Kochansky, John R. Strauss, Ronald B. Franklin, and Stella H. Vincent

Departments of Drug Metabolism (M.-S.K., S.W., Z.S., C.J.K., R.B.F., S.H.V.) and Comparative Medicine (J.R.S.), Merck Research Laboratories, Rahway, New Jersey

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ABSTRACT:

Genetically obese Zucker rats exhibit symptoms similar to those of obese patients with insulin-resistance or Type II diabetes; therefore, they have been used as a genetic model to study obesity, as well as a pharmacological model for the discovery of new drugs for the treatment of Type II diabetes and hyperlipidemia. In the present study, we compared the pharmacokinetics of two novel peroxisome proliferator-activated receptor (PPAR) agonists, MRL-I ([2R]-7-[2-chloro-4-(4-fluorophenoxy)phenoxy]propoxy)-2-ethyl-3,4-dihydro-2H-benzopyran-2-carboxylic acid) and MRL-II ([2R]-7-[2-chloro-4-(2,2,2-trifluoroethoxy)phenoxy]propoxy)-3,4-dihydro-2-methyl-2H-benzopyran-2-carboxylic acid), in obese Zucker and lean Sprague-Dawley rats following a single intravenous administration. The plasma clearance of both MRL-I and MRL-II was significantly lower in obese Zucker rats (4- and 2-fold, respectively) compared with Sprague-Dawley rats, but without any significant change in the volume of distribution, which resulted in a dramatic increase in the half-life (7- and 3-fold, respectively). The reversible in vitro plasma protein binding of [14C]MRL-I and [14C]MRL-II was comparable in the two strains, ~96% bound. The expression levels of uridine diphosphate-glucuronosyltransferases 1A1, 1A6, 2B1, and CYP2C11 and 3A1 mRNA in liver were lower (30-50%) in Zucker compared with Sprague-Dawley rats, as were the liver glutathione S-transferases (70%), quinone reductase (30%), organic anion-transporting protein 2 (80%), and multidrug resistance-associated protein 2 (Mrp2) (50%) mRNA levels. However, Mrp3 mRNA levels were similar in both strains. Consistent with these observations, the intrinsic clearance (CLint), calculated from the Vm/Km of glucuronidation of [14C]MRL-I and [14C]MRL-II in liver microsomes, was ~2-fold lower in obese Zucker rats; the Kms values were comparable in the two strains for both compounds. In conclusion, differences in the pharmacokinetics of two novel PPAR agonists, both cleared, predominantly, by conjugation, were evident in genetically obese Zucker rats compared with Sprague-Dawley rats. These differences were consistent with changes in the mRNA levels of hepatic drug-metabolizing enzymes and transporters. This information should be considered when comparing pharmacokinetic and efficacious doses in the obese Zucker rats, used as a pharmacological model, with those in Sprague-Dawley rats, which are used widely for drug metabolism and toxicology studies.

The genetically obese Zucker rat arose from cross-breeding between Sherman and Merck stock M rats and obesity is transmitted as a Mendelian recessive trait due to a mutation on a single gene, called “fa”, for “fatty” (Zucker and Zucker, 1961). The symptoms of this genetic obesity share many similarities with those in obese patients with insulin-resistance or Type II diabetes. Therefore, the obese Zucker rat has been widely used as a genetic model of obesity and insulin resistance (Bray, 1977; Kurtz et al., 1989; Krief and Bazin, 1991).

Synthetic agonists for the peroxisome proliferator-activated receptors (PPAR) α (fibrates) and PPAR γ (thiazolidinediones) are widely used in the treatment of dyslipidemia and diabetes, respectively (Wahl et al., 1995; Schoonjans et al., 1996; Kersten et al., 2000; Berger and Moller, 2002). MRL-I, (2R)-7-[2-chloro-4-(4-fluorophenoxy)phenoxy]propoxy]-2-ethyl-3,4-dihydro-2H-benzopyran-2-carboxylic acid (Fig. 1), is a PPAR α/γ dual agonist that is structurally distinct from marketed PPAR agonists. Its plasma glucose-lowering efficacy in db/db mice is similar to that of rosiglitazone, whereas its lipid-lowering efficacy is better than that of fibrates (Koyama et al., 2004). MRL-II, (2R)-7-[2-chloro-4-(2,2,2-trifluoroethoxy)phenoxy]propoxy]-3,4-dihydro-2-methyl-2H-benzopyran-2-carboxylic acid (Fig. 1), is chemically similar to MRL-I but

ABBREVIATIONS: PPAR, peroxisome proliferator-activated receptor; CLint, intrinsic clearance; P450, cytochrome P450; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; Mrp, multidrug resistance; MRL-I, (2R)-7-[2-chloro-4-(4-fluorophenoxy)phenoxy]propoxy]-2-ethyl-3,4-dihydro-2H-benzopyran-2-carboxylic acid; MRL-II, (2R)-7-[2-chloro-4-(2,2,2-trifluoroethoxy)phenoxy]propoxy]-3,4-dihydro-2-methyl-2H-benzopyran-2-carboxylic acid; Mrp, multidrug resistance protein; Oatp, organic anion-transporting polypeptide; UDPGA, uridine diphosphate-glucuronic acid; UGT, uridine diphosphate-glucuronosyltransferase.
has PPAR α-selective agonist properties. The cholesterol- and triglyceride-lowering potency of MRL-II was shown to be superior to that of fenofibrate in various in vivo models (H. Koyama, J. K. Bouveres, D. J. Miller, J. P. Berger, K. L. MacNaul, L. J. Kelly, T. W. Doebber, P. R. Wang, M. C. Ippolito, Y.-S. Chao, et al., manuscript in preparation).

Previous work has shown altered pharmacokinetic characteristics of some drugs in obese subjects (Abernethy and Greenblatt, 1982); however, the relevance of clinical obesity to drug metabolism, disposition, and excretion is poorly characterized. Pharmacokinetic differences between obese Zucker and lean Sprague-Dawley rats also have been reported with, for example, phenobarbital (Brouwer et al., 1984), but no explanation for this observation was provided.

In the present study, we compared the pharmacokinetics of MRL-I and MRL-II in obese Zucker and Sprague-Dawley rats. The former strain of rats is used as an animal model for obesity and diabetes and the latter strain is used commonly in drug metabolism and toxicology studies. To begin to address the possible underlying mechanisms behind differences in the pharmacokinetics of xenobiotics between these two strains, the in vitro kinetics of glucuronidation and plasma protein binding of MRL-I and MRL-II in obese Zucker and Sprague-Dawley rats were evaluated. In addition, the mRNA levels of xenobiotic transporters and hepatic drug-metabolizing enzymes in these strains were determined.

Materials and Methods

Chemicals. MRL-I, [ethyl-14C]MRL-I, MRL-II, [13CD]MRL-II, and [methyl-14C]MRL-II (Fig. 1) were synthesized at Merck Research Laboratories and were provided as their arginine salts. Alamethicin, saccharolactone, and UDPGA were purchased from Sigma-Aldrich (St. Louis, MO). Acetoni- trile and methanol (HPLC grade) were obtained from Fisher Scientific Co. (Pittsburgh, PA). All other reagents were of analytical or HPLC grade.

Animals. Male Sprague-Dawley and obese Zucker rats were obtained from Charles River Laboratories Inc. (Wilmington, MA). They were housed in cages and maintained on a 12-h light/dark cycle. Access to food and water was allowed ad libitum. Rats used in this study were 14 to 16 weeks old; the Sprague-Dawley rats weighed ~300 g and the obese Zucker rats weighed ~600 g. All procedures for animal experiments were approved by the Merck Research Laboratories Institutional Animal Care and Use Committee.

Studies in Bile Duct-Cannulated Rats. [14C]MRL-I (1 mg/kg) or [14C]MRL-II (0.5 mg/kg) was dosed intravenously by a bolus injection into the femoral vein of male Sprague-Dawley rats whose bile ducts had been surgically cannulated (n = 3 or 4). Each rat was given ~25 to 30 μCi of radioactivity. Bile and urine were collected for up to 72 h postdose at specific time intervals into bottles containing 0.5 M formate buffer, pH 3.0, to stabilize labile acyl glucuronide metabolites. Bile was collected at the following time intervals: predose, 0 to 1, 1 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 24, 24 to 48, and 48 to 72 h. Urine was collected at 24-h intervals up to 72 h postdose. Levels of radioactivity in the bile and urine were determined by liquid scintillation counting (Beckman LS 6500; Beckman Coulter, Fullerton, CA).

Metabolite Identification. Bile samples from the rats dosed with 1 mg/kg [14C]MRL-I were treated with an equal volume of acetonitrile, spun in a centrifuge at 16,000g for 10 min, and the supernatants were analyzed using an HPLC system consisting of a Shimadzu (Kyoto, Japan) series 200 pump, a Shimadzu series 200 autosampler, and a β-RAM scintillation detector (INUS Systems, Inc., Tampa, FL). The samples were chromatographed on a 3.5-μm Zorbax RX-C18 column (150 × 4.6 mm; Agilent Technologies, Palo Alto, CA). The mobile phase consisted of 10 mM ammonium acetate in water (A) and 7.8 mM ammonium acetate in 92:8 acetonitrile/methanol, v/v (B), and the flow rate was 1 ml/min. The column was eluted with a linear gradient from 25 to 60% B over 30 min, followed by a linear gradient to 90% B in 1 min, and isocratic elution at 90% B for 5 min. Identification of [14C]MRL-I metabolites was achieved by LC-MS/MS using a PerkinElmerSciex Instruments (Boston, MA) API 3000 triple quadrupole mass spectrometer. The Turbo Ionspray source was operated in the negative ion mode at 400°C with an auxiliary nitrogen gas flow of 7.0 l/min. The HPLC effluent was split 1 to 4, with 0.2 ml/min directed into the mass spectrometer and the remaining 0.8 ml/min directed to a radioactivity flow-through detector (β-RAM; INUS Systems, Inc.).

Identification of metabolites in bile samples from studies with [14C]MRL-II was achieved by HPLC analysis of acetonitrile extracts and comparison of retention times with chemically synthesized standards. Radiochromatograms were obtained using an on-line PerkinElmer Flow Scintillation Analyzer (PerkinElmer Life and Analytical Sciences, Boston, MA) at room temperature. Separation of metabolites was achieved using a PerkinElmer 200 series HPLC system equipped with a 5-mm Betasil Phenyl column (250 × 4.6 mm; Thermo Hypersil, Keystone Scientific Operations, Bellefonte, PA). The mobile phase consisted of 1 mM ammonium acetate in water/acetonitrile/acetic acid (95:5:0.1, by volume) (A) and 1 mM ammonium acetate in acetonitrile/water/acetic acid (95:5:0.1, by volume) (B). Separation was achieved under isocratic conditions from 0 to 5 min at 10% B, followed by a 5-min linear gradient to 45% B, an isocratic hold for 10 min at 45% B, and then a linear gradient to 55% B in 10 min and to 95% B in 4 min, followed by a 7-min wash with 95% B. The flow rate was 1 ml/min.

In Vivo Pharmacokinetic Studies. Rats were anesthetized by intramuscular administration of a mixture consisting of 4 ml of ketamine (100 mg/ml), 2 ml of xylazine (20 mg/ml) and 1 ml of atropine (1 mg/ml), at 100 μl/100 g of body weight, and their femoral arteries and veins were cannulated surgically. After a full day of recovery from surgery, MRL-I or MRL-II in 0.9% saline was dosed intravenously via the femoral vein, at 1 or 2 mg/kg, respectively. Blood was drawn from the femoral artery into heparinized containers at regular intervals up to 48 h postdose. Plasma was obtained by centrifugation at 1600g for 10 min, acidified with 0.5 M formate buffer, pH 3.0 (300 μl buffer/ml plasma), and stored at ~70°C until analysis by LC-MS/MS.

Quantification of MRL-I in Rat Plasma. Concentrations of MRL-I in rat plasma were determined by LC-MS/MS following acetonitrile precipitation of plasma proteins. Briefly, 65-μl aliquots of acidified plasma (corresponding to 50 μl of nonacidified plasma) were mixed with internal standard, (2R)-7-[3-[2-chloro-4-(4-phenoxy)phenoxo]propoxy]-2-ethyl-3,4-dihydro-2H-benzopyran-2-carboxylic acid, followed by the addition of 400 μl of acetonitrile. Samples were then spun in a centrifuge at 1600g for 10 min, and supernatants were transferred by a Tomtec unit (Qudra 96, model 320; Tomtec, Hamden, CT) and subjected to LC-MS/MS analysis. The LC-MS/MS system consisted of a PerkinElmer HPLC system and a PerkinElmerSciex API 3000 tandem mass spectrometer operated in the negative ionization mode using a Turbo
Ionspray. Multiple reaction monitoring of the following precursor→product ion combinations was used for detection of analytes: MRL-I, m/z 499.5→237 and internal standard, m/z 481.4→219. The HPLC column (BetaBasic 18, 50×2 mm; Thermo Hypersil, Keystone Scientific Operations) was eluted with 70% A (1 mM ammonium acetate) and 30% B (acetonitrile) for 0.4 min, followed by a linear gradient to 90% B in 1 min, and a 1.5-min hold at 90% B with a flow rate of 0.2 ml/min. The analytical detection limit was 5 ng/ml.

**Quantification of MRL-II in Rat Plasma.** Concentrations of MRL-II in rat plasma were determined by LC-MS/MS as described above for MRL-I except that [14C]MRL-II was used as the internal standard. Multiple reaction monitoring was used to detect the analytes, using the following precursor→product ion combinations: MRL-II, m/z 473.4→225.1 and [14C]MRL-II, m/z 477.3→142.2. Samples were injected onto an ACE 5 phenyl column (100×2.1 mm, 5 μm; MAC-MOD Analytical, Inc., Chadds Ford, PA) and eluted with a mobile phase consisting of 1 mM ammonium acetate and 0.002% formic acid in water (A) and acetonitrile (B). Separation was achieved at 0.2 ml/min with a linear gradient of 30 to 95% B over 4 min and a 1-min hold at 95% B. The analytical detection limit was 5 ng/ml.

**Determination of Pharmacokinetic Parameters.** Concentrations of the analytes were computed from the peak area ratios (relative to internal standard) using external calibration curves. The pharmacokinetic parameters were calculated using Watson software (version 6; Watson Software Systems) or Microsoft Excel v 97 SR-1 (Microsoft, Redmond, WA) for noncompartmental models.

**Determination of Reversible Plasma Protein Binding.** Fresh EDTA-treated blood was collected from three male Sprague-Dawley and five male obese Zucker rats. Plasma was obtained by centrifugation at 1600g for 10 min. Aliquots of rat plasma (0.5 ml) were mixed with aqueous solutions of [14C]MRL-I or [14C]MRL-II to a final concentration of 1 μM. The resulting plasma samples were transferred into one side of dialysis cells (Bel-Art Products, Pequannock, NJ) which were separated by a dialysis membrane (Sigma-Aldrich) with a molecular weight cutoff of 12,400, and were dialyzed against isotonic phosphate buffer for 24 h at 37°C. The unbound fraction was determined by comparing the amount of radioactivity in 100-μl aliquots of buffer and plasma, determined by liquid scintillation counting.

**Evaluation of the Expression of Xenobiotic Transporters and Hepatic Drug-Metabolizing Enzymes.** The mRNA levels for several drug-metabolizing enzymes, including rat P450s, UGTs, and hepatic transporters for which specific oligonucleotide probes were available at Merck Research Laboratories, were evaluated in Sprague-Dawley and obese Zucker rats. Specific primers and probes for quantitative reverse transcription-polymerase chain reaction were developed as described by Wang et al. (2003). Total RNA was isolated from Sprague-Dawley and Zucker rat livers using the SV Total RNA Isolation System (Promega, Madison, WI) according to the manufacturer’s instructions. Samples were quantitated by spectrophotometry and diluted to a concentration of 15 μg/ml. RNA integrity was checked by agarose/formaldehyde gel electrophoresis. Samples were then assayed in triplicate 25-μl reactions using 25 ng of RNA per reaction. Gene-specific primers were used at 7.5 μM (data not shown). From the results of in vivo studies, there was no indication of instability of the acyl glucuronide conjugate was the major radioactive component detected in bile from rats dosed with [14C]MRL-I and [14C]MRL-II, whereas bile samples from rats dosed with [14C]MRL-I also contained the taurine conjugate of parent compound (Fig. 2).

**Pharmacokinetics of MRL-I and MRL-II.** Table 2 summarizes the pharmacokinetic parameters of MRL-I and MRL-II in Sprague-Dawley and obese Zucker rats following a single intravenous dose. The plasma clearance was lower (4-fold and 2-fold, respectively, for MRL-I and MRL-II) and the half-life was longer (7- and 3-fold, respectively) in obese Zucker rats compared with Sprague-Dawley rats, without any significant differences in the volume of distribution.

**Reversible Plasma Protein Binding.** The protein binding of [14C]MRL-I and [14C]MRL-II was investigated in plasma from Sprague-Dawley and obese Zucker rats. The free fraction of [14C]MRL-I and [14C]MRL-II was ~3.9 and 4.2%, respectively, in both obese Zucker and Sprague-Dawley rats at a concentration of 1 μM. Plasma protein binding in Sprague-Dawley rats was not concentration-dependent between 1 and 50 μM (data not shown). From the results of in vivo studies, there was no indication of instability of [14C]MRL-I and [14C]MRL-II in plasma at 37°C.

**Expression of Hepatic Drug-Metabolizing Enzymes and Xenobiotic Transporters.** The mRNA levels (normalized relative to

<table>
<thead>
<tr>
<th>Samples</th>
<th>MRL-I</th>
<th>MRL-II</th>
</tr>
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<tbody>
<tr>
<td>Bile</td>
<td>102 ± 10.4a</td>
<td>62 ± 3a</td>
</tr>
<tr>
<td>Urine</td>
<td>8.2 ± 2.7</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>Total</td>
<td>110 ± 13.1</td>
<td>91 ± 0.1</td>
</tr>
</tbody>
</table>

a Bile and urines samples were collected over a 72-h period.

b Mean ± standard deviation values are from n = 4 or 3 male rats for MRL-I and MRL-II, respectively.
Zucker rat has been used as a genetic model to study obesity, as well as the clinical observations in obese patients with Type II diabetes, the symptoms of obesity, hyperglycemia, hyperinsulinemia, and hyperlipidemia (Stevenson and Kaysen, 1999). Since these symptoms are similar to those of obesity, hyperglycemia, hyperinsulinemia, and hyperlipidemia as a pharmacological model for the discovery of new drugs for the treatment of Type II diabetes and hyperlipidemia (Bray, 1977; Kurtz et al., 1989; Krief and Bazin, 1991).

In the studies reported herein with the PPAR agonists MRL-I and MRL-II, we have observed significant differences in their pharmacokinetics in genetically obese Zucker and lean Sprague-Dawley rats (Table 1). Previously, Brouwer et al. (1984) reported different pharmacokinetics of phenobarbital in the Zucker and Sprague-Dawley rat. After a single intravenous bolus administration, the plasma clearance of phenobarbital in the obese Zucker and lean Sprague-Dawley rats was 20 and 56 ml/h/kg, respectively, whereas the volume of distribution was comparable. However, no explanation was provided for these observations. Since the data from the present study were in agreement with those of Brouwer et al. (1984), we have proceeded to investigate the possible underlying mechanisms that caused these differences in the pharmacokinetics of MRL-I and MRL-II.

TABLE 2
Pharmacokinetic parameters of MRL-I and MRL-II following intravenous administration to male Sprague-Dawley and obese Zucker rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MRL-I</th>
<th>MRL-II</th>
</tr>
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<tbody>
<tr>
<td>Sprague-Dawley Obese Zucker</td>
<td>Sprague-Dawley Obese Zucker</td>
<td></td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AUC (µM · h)</td>
<td>9.9 ± 1.5</td>
<td>40 ± 12</td>
</tr>
<tr>
<td>CLm (ml/min/kg)*</td>
<td>3.4 ± 0.5</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td>Va (l/kg)</td>
<td>0.8 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>t1/2 (h)*</td>
<td>3.4 ± 0.7</td>
<td>24 ± 9.3</td>
</tr>
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</table>

*Significantly different between obese Zucker and Sprague-Dawley rats (p < 0.005, Student’s t test).

In Vitro Glucuronidation. The UGT-mediated metabolism of MRL-I and MRL-II was evaluated in liver microsomes prepared from Sprague-Dawley and obese Zucker rats. The kinetic parameters Vmax and Km are summarized in Table 4. The Vmax/Km values, calculated from Vmax/Km, were ~2-fold higher in Sprague-Dawley rat liver microsomes, whereas the Km values were comparable for both compounds.

After a single intravenous bolus administration, the plasma clearance of phenobarbital in the obese Zucker and lean Sprague-Dawley rats was 20 and 56 ml/h/kg, respectively, whereas the volume of distribution was comparable. However, no explanation was provided for these observations. Since the data from the present study were in agreement with those of Brouwer et al. (1984), we have proceeded to investigate the possible underlying mechanisms that caused these differences in the pharmacokinetics of MRL-I and MRL-II.

Fig. 2. Metabolite profiles of bile following intravenous administration of [14C]MRL-I and [14C]MRL-II to male Sprague-Dawley rats at 1 and 0.5 mg/kg, respectively. Samples from individual animals (n = 4 or 3 for MRL-I and MRL-II, respectively) were pooled based on the volume collected at different time points (0–72 h) and pooled again by the same volume across animals.
concentrations of α1-acid glycoprotein in the obese human were
double those of normal-weight controls, resulting in increased serum
protein binding of propranolol. Similarly, the plasma protein binding
of propranolol and diazepam was higher in obese than in lean Zucker
rat. In contrast, the binding of phenytoin, which is primarily bound to
serum albumin, was lower in obese Zucker rats (Porter and Layzer,
1975). Based on the report on the serum chemistry by Benedek et al.
(1985), albumin concentrations were comparable among obese
Zucker, lean Zucker, and Sprague-Dawley rats. However, the concen-
trations of free fatty acid, and cholesterol and triglyceride levels were
higher in obese Zucker rats compared with the lean Zucker and
Sprague-Dawley rats, which may affect drug-serum binding. Serum
free fatty acids have been reported to displace phenytoin from rat
albumin binding sites (Colburn and Gibaldi, 1978). Therefore, phe-
nytoin displacement by elevated serum free fatty acids is most likely
the reason for the decreased binding of phenytoin to serum albumin.
Also, the presence of increased lipoprotein and other serum protein
concentrations appeared to be responsible for the significant increase
of protein binding of diazepam in obese compared with lean Zucker
rats (Benedek et al., 1985).

In light of this information, the reversible plasma protein binding of
[MRL-I] and [MRL-II] was evaluated in Sprague-Dawley and
obese Zucker rats. The present data indicated that altered protein
binding was not one of the contributing factors to the different
pharmacokinetics with MRL-I and MRL-II.

In Sprague-Dawley rats, MRL-I and MRL-II were shown to be
eliminated primarily by glucuronidation of the carboxylic acid moiety,
followed by excretion of the resulting acyl glucuronide predominately
into the bile (Table 1; Fig. 2). The rat UGTs catalyzing MRL-I and
MRL-II have not been characterized; however, in vitro studies using
baculovirus-expressed human UGT Supersomes indicated that MRL-I
was a good substrate of UGT1A3 and, to a lesser extent, UGT1A6,
whereas UGTs1A3, 1A9, and 2B7 exhibited similar
activity toward MRL-II, with UGT1A1 and 1A8 showing a much
lower extent of glucuronidation (Kim et al., 2004). In light of this
information, the expression of rat hepatic UGTs, 1A1, 1A6, and 2B1,
in obese Zucker and Sprague-Dawley rats was evaluated. The results
indicated that the mRNA expression of the UGTs was lower in
Zucker than in Sprague-Dawley rats. Similarly, lower mRNA levels
were observed for CYP3A1, CYP2C11, glutathione S-transferase, and
quino-none reductase. The activities of UGTs in both strains were further
characterized in the in vitro glucuronidation kinetic studies. The CLint,
calculated from V_{max}/K_{m} in liver microsomes of Sprague-Dawley
rats, was ~2-fold higher than that in obese Zucker rat liver micro-
somes, consistent with the observed differences in plasma clearance
between the two strains (Table 3). The intrinsic clearance of
[MRL-I] was higher than that of [MRL-II], especially in
Sprague-Dawley rats. In contrast, the plasma clearance of MRL-I was
the same as that of MRL-II in Sprague-Dawley rats. These results are
consistent with the observation that MRL-II is eliminated not only by
acyl glucuronidation but also by taurine conjugation after intravenous
administration (Table 1; Fig. 2).

Altered metabolism in hepatic microsomes and cytosol from genet-
ically obese Zucker rats has been reported previously. The specific
activities of P450 and glutathione S-transferase in obese Zucker rats
were 50 to 70% lower than those in lean Zucker rats and Sprague-
Dawley rats (Litterst, 1980). Additionally, altered expression of he-
catic CYP2E1 and CYP4A in obese, diabetic ob/ob, and fa/fa Zucker
rats has been reported (Enriquez et al., 1999; Roe et al., 1999). The
data showing lower hepatic xenobiotic enzyme activities in geneti-
cally obese rats can be contrasted with the data reported for acetamino-
phen by Wong et al. (1986) in diet-induced obese rats, and by
(1986) have shown enhanced glucuronidation and sulfate conjugation
of acetaminophen in diet-induced obese rats. Chaudhary et al. (1993)
reported a higher rate of acetaminophen glucuronidation in liver
microsomes from obese Zucker rats than from lean control rats, but
the rates of glucuronidation of 1-naphthol, estrone, and morphine
were comparable between lean and obese Zucker rats. The reason(s)
for these apparent discrepancies remains to be characterized; however,
based on the report by Chaudhary et al. (1993), the effect of genetic
obesity on glucuronidation appears to be substrate-specific. It is also
possible that physiological differences, such as age, and environmen-
tal factors may be responsible for these differences. Galinsky et al.
(1986) reported that the fraction excreted as the glucuronide and the
partial clearance of acetaminophen glucuronidation increased with
age. The animals used in our study were 14 to 16 weeks old and were
relatively younger than the 5- to 6-month-old animals used by
Chaudhary et al. (1993) in their study. Both multidrug resistance
(mdr) knockout mice housed in Amsterdam had in-
creased levels of CYP3A protein, whereas the hepatic P450 expres-
sion was unaffected or decreased in the genetically identical
mdr1a and mdr1b knockout mice housed in the United States (Schuetz
et al., 2000), suggesting that the expression of hepatic drug-metaboliz-
ing enzymes depends not only on the nature of the genetics but also on
the environmental factors. Interestingly, enhanced glucuronidation of
acetaminophen, as well as lorazepam and oxazepam, has been
reported in obese humans (Abernethy and Greenblatt, 1982). Again,
the reasons for this apparent discrepancy in glucuronidation between
genetically obese Zucker rats and obese humans remains to be deter-
mined; however, different genetics leading to differences in physiol-
ogy may be a contributing factor. The obesity, hyperglycemia, and
hyperinsulinemia observed in obese Zucker rats are the result of a
mutation on the leptin receptor (Campfield et al., 1996). However,
human obesity is not believed to be related to leptin deficiency
(Considine, 1996).

Rat Oatp2, a member of the multispecific organic anion-transport-
ing polypeptide family (Noe et al., 1997; Abe et al., 1998), is ex-
pressed in the hepatic sinusoidal membrane and it transports anions,
cations, and neutral compounds (Abe et al., 1998; Eckhardt et al.,

**TABLE 4**

<table>
<thead>
<tr>
<th>Kinetic Parameters</th>
<th>MRL-I</th>
<th></th>
<th>MRL-II</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Sprague-Dawley</td>
<td>Obese Zucker</td>
<td>Sprague-Dawley</td>
<td>Obese Zucker</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V_{max} (nmol/min/mg)*</td>
<td>0.73 ± 0.08</td>
<td>0.45 ± 0.02</td>
<td>1.28 ± 0.03</td>
<td>0.73 ± 0.12</td>
</tr>
<tr>
<td>K_{m} (µM)</td>
<td>15.2 ± 1.7</td>
<td>21.0 ± 2.8</td>
<td>48.4 ± 2.5</td>
<td>53.5 ± 15.9</td>
</tr>
<tr>
<td>CL_int (µl/min/mg)*</td>
<td>47.7 ± 4.2</td>
<td>21.4 ± 2.4</td>
<td>26.6 ± 0.8</td>
<td>14.1 ± 2.1</td>
</tr>
</tbody>
</table>

* Significantly different between obese Zucker and Sprague-Dawley rats (p < 0.05, Student’s t test).
Mrp2 is a transporter that was originally recognized in rat hepatocyte canalicular membranes and has been shown to mediate the ATP-dependent transport of glutathione, glucuronide, and sulfate conjugates of lipophilic compounds, and several other endogenous and xenobiotic compounds (Jansen et al., 1985; Oude Elferink and Jansen 1994; Gerk and Vore, 2002). Another organic anion transporter, Mrp3, is expressed almost exclusively in the intestine, with much lower levels found in other tissues in Sprague-Dawley rats (Cherrington et al., 2002). This transporter is localized to the basolateral membrane where it is involved in the excretion of various organic anions from cells into the sinusoidal blood (Hiroi et al., 1999; Renes et al., 2000).

In the present studies, it was determined that the expression of oatp2 and, to a lesser extent, Mrp2 was lower in obese Zucker rats than in Sprague-Dawley rats. Both transporters are believed to play major roles in the disposition of organic anions, catalyzing, respectively, their initial uptake from blood into hepatocytes (Nee et al., 1997; Abe et al., 1998) and subsequent secretion into bile across the canalicular membrane (Gerk and Vore 2002; Kim et al., 2003). Thus, the lower mRNA levels of oatp2 in obese Zucker rats may have resulted in decreased sinusoidal hepatic uptake of MRL-I and MRL-II, as compared with Sprague-Dawley rats, and may, in part, be responsible for their lower plasma clearance. Similarly, since hepatic biliary excretion as acyl glucuronides is the major elimination pathway for both MRL-I and MRL-II, it is conceivable that the decrease in the expression of Mrp2 could have affected the biliary clearance of MRL-I and MRL-II. Additional studies are needed to determine definitively whether the decreased expression of oatp2 and Mrp2 in Zucker rats may have contributed to the decreased clearance of MRL-I and MRL-II compared with Sprague-Dawley rats.

In summary, we have demonstrated that the pharmacokinetics of MRL-I and MRL-II, compounds cleared principally by phase II metabolism, were significantly altered in genetically obese Zucker rats compared with Sprague-Dawley rats. Furthermore, differences in the mRNA levels of phase I and phase II hepatic drug-metabolizing enzymes and transporters were documented and may have contributed to the observed differences in pharmacokinetics between the two strains of rats.

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Address correspondence to: Dr. Mi-Sook Kim, Department of Drug Metabolism, Merck Research Laboratories, P.O. Box 2000, RY80L-109, Rahway, NJ 07065. E-mail: misook_kim@merck.com

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