Metabolism and Disposition of 14C-Labeled Peliglitazar in Humans

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ABSTRACT:

The metabolism and disposition of dual 14C-labeled peliglitazar, a dual α/γ peroxisome proliferator-activated receptor activator, was investigated in 10 healthy male subjects with and without bile collection (groups 1 and 2) after a single 10-mg oral dose. Serial blood samples, urine, and feces (0–240 h) as well as bile samples (3–8 h after dosing from group 2 subjects) were collected. The maximum plasma concentration (C_max) of drug was reached at approximately 1 h and the elimination half-life (t_1/2) was approximately 3.5 h. The exposure to drug metabolites (C_max and area under the plasma concentration versus time curve) was not significantly different between the two groups. The parent compound and its 1-O-β-acyl-glucuronide conjugate were the major components in plasma; other circulating metabolites, including several other glucuronide conjugates, were minor components at all time points. The major portion of the radioactive dose was recovered in feces (94% for group 1 and 32% for group 2). Approximately 24% of the radioactive dose was recovered in the bile from group 2 subjects, nearly all of which was assigned as glucuronides of peliglitazar and its oxidative metabolites (M14, M14a, M14b, M15, M15a, M15b, and M17). In contrast, fecal samples contained peliglitazar and its oxidative metabolites resulting from aliphatic/aryl hydroxylation, and O-demethylation. These results suggested that the major clearance pathway of peliglitazar was through biliary elimination of glucuronide conjugates, which were hydrolyzed to peliglitazar and its oxidative metabolites in the intestines before excretion.

Introduction

Peroxisome proliferator-activated receptors (PPARs) are a set of nuclear hormone receptors (comprising the α, γ, and δ subtypes) that act as transcription factors in the regulation of multiple genes involved in diverse disease areas such as type 2 diabetes, dyslipidemia, obesity, inflammation, cancer, and osteoporosis (Torra et al., 2001; Taskinen, 2003; Yajima et al., 2004). The two most intensively investigated subtypes have been PPARα (which is primarily expressed in the liver and plays a critical role in lipid metabolism) and PPARγ (which is predominantly expressed in adipose tissue and is implicated in insulin sensitization as well as glucose and fatty acid utilization). PPARα is the target of the fibrate class of hypolipidemic drugs such as fenofibrate (Balfour et al., 1990; Després, 2001; Packard et al., 2002) and gemfibrozil (Spencer and Barradell, 1996), whereas PPARγ is the target of the thiazolidinedione (Mudaliar and Henry, 2001) class of antidiabetic drugs such as rosiglitazone (Balfour and Plosker, 1999; Després, 2001; Packard et al., 2002) and pioglitazone (Gil-Grande and Dunn, 2000).

Materials and Methods

Radiolabeled Drug and Chemicals. Two versions of 14C-labeled peliglitazar (Fig. 1) were synthesized separately at Bristol-Myers Squibb (Princeton, NJ) and mixed at a 1:1 ratio as a dual radiolabeled material. The final specific activity of [14C]peliglitazar was 10.26 μCi/mg (99.6% radiochemical purity). The reference compounds for metabolites M1, M2, M3, M4, M5, M7, and M14 were synthesized at Bristol-Myers Squibb using procedures similar to those described previously (Wang et al., 2006; Zhang et al., 2006), and their LC-MS/MS spectra are shown in the supplemental data. Trifluoroacetic acid and chemical reagents for synthesis were purchased from Aldrich Chemical Co. (Milwaukee, WI). Ecolite liquid scintillation cocktail and acetonitrile were purchased from ICN Biomedicals Inc. (Costa Mesa, CA). All other organic solvents and reagents were of HPLC grade.

Human Studies. The study was performed in accordance with the following codes and guidelines: Title 21, Part 56 CFR (Institutional Review Board Approval); Title 21, Part 50 CFR (Protection of Human Subjects); the princi-
Ten healthy male subjects, aged 18 to 45 years, were assigned to two groups, groups 1 (six subjects) and group 2 (four subjects). After at least an 8-h overnight fast, each subject (groups 1 and 2) received a single dose of 10 mg of [14C]peliglitazar containing 102.6 Ci of radioactivity as an oral solution in PEG-400. All subjects remained in the clinical facility for 10 days and were closely monitored for adverse events throughout the study. A single oral cathartic dose of milk of magnesia (30 ml) was administered on the evening of day 7, to ensure defecation before release from the clinical facility. Subjects were discharged from the clinic in the afternoon of day 11 provided that the day 8 measurement of radioactivity in feces was ≥1% of the administered radioactivity.

Collection of Blood, Bile, Urine, and Feces. Blood samples for pharmacokinetic analysis were drawn (5 ml each) before dosing and at 0.25, 0.5, 1, 1.5, 2, 4, 8, 12, 24, 48, 72, 96, and 120 h postdose. Blood samples (10 ml each) for biotransformation analysis were drawn at 1, 4, 12, 24, and 48 h postdose. The blood samples were collected by direct venipuncture using Vacutainers (Beckman Coulter, Fullerton, CA). Liquid scintillation counter data were automatically corrected for counting efficiency using an external standardization technique and an instrument-stored quench curve generated from a series of sealed quench standards. The volume of bile and urine and weight of feces collected over each interval and the concentrations of radioactivity in the corresponding samples were used to calculate the cumulative percentage of the administered dose recovered in the bile, urine, and feces for the estimation of biliary, urinary, and fecal excretion of radioactivity.

Pharmacokinetic Analyses of Radioactivity. The pharmacokinetic parameters of the plasma radioactivity were analyzed by a noncompartmental method (Gibaldi and Perrier, 1982). The peak plasma concentration, C_{max}, and the time to reach peak concentration, T_{max}, were recorded directly from experimental observations. The area under the plasma concentration versus time curve (AUC) was calculated by a combination of the trapezoidal and log-trapezoidal methods. The AUC was calculated from time 0 to 12 h. The first-order rate constant of decline of radioactivity concentration, expressed as a half-life of peliglitazar, in the terminal phase of each plasma concentration versus time profile, K, was estimated by log-linear regression (using no weighting factor) of at least three data points, which yielded a minimum mean square error. The absolute value of K was used to estimate the apparent terminal elimination half-life, t_{1/2}. The last measurable concentration and K were used to extrapolate the AUC_{0–12 h} to estimate AUC_{0–INF}.

Sample Preparation for Biotransformation Analysis. Pooled plasma samples (at 1, 4, 12, and 24 h) from each group were prepared separately by mixing an equal volume (0.5 ml) of plasma sample from each subject. The plasma samples at 48 h were not analyzed because of low levels of radioactivity. Portions (1 ml) of the pooled plasma samples were extracted by addition of a mixture of 1 ml of methanol and 3 ml of acetonitrile and mixed on a vortex mixer. The mixtures were centrifuged at 2000 g for 30 min, and then the supernatants were transferred into a polypropylene centrifuge tube. The extractions were repeated two more times, and all supernatants were combined. The radioactivity recovery of the extraction was calculated on the basis of the radioactivity determination before and after extraction. The combined supernatants were concentrated under a stream of nitrogen. The residues were reconstituted in 0.5 ml of a solution of 70% of HPLC mobile phase A (0.06% TFA in water) and 30% mobile phase B (0.06% TFA in acetonitrile), vortexed, and centrifuged at 2000 g for 10 min, and 100 μl of the supernatant was used for the HPLC analysis.

A grand pool of bile samples was prepared by mixing 3% of bile by volume from each collection interval of all subjects. The individual pooled bile samples were prepared by mixing 1% of bile by volume from each collection interval of each subject. The interval pooled bile samples (3–4, 4–6, and 6–8 h) were prepared by mixing 1% of bile by volume from each collection interval of each subject. A portion (0.1 ml) of each pooled bile sample was diluted 10 times with HPLC mobile phase A, and 150 μl of the diluted sample was used for the HPLC analysis.

Two grand pools of urine samples (0–240 h) from group 1 and 2 were prepared separately by mixing 1% of urine by weight of each collection from all subjects in each group. A portion (30 ml) of the 1% grand pooled urine samples (0–240 h) from each group was concentrated under a steam of nitrogen. The residues were reconstituted with 1.0 ml of a solution of 70% of HPLC mobile phase A and 30% mobile phase B, vortexed, and centrifuged at 2000 g for 10 min, and 200 μl of the supernatant was used for HPLC analysis.

Two grand pools of fecal homogenates (0–240 h) from group 1 and 2 were prepared separately by mixing 1% of homogenates by weight of each
collection from all subjects in each group. The grand pooled fecal homogenates (1.0 ml) were extracted by addition of a mixture of 1 ml of methanol and 3 ml of acetonitrile and mixed on a vortex mixer. The mixtures were sonicated for 10 min then centrifuged at 2000g at 10°C for 30 min, and the supernatants were transferred into a polypropylene centrifuge tube. The extraction was repeated one more time, and all supernatants were combined. The radioactivity recovery of the extraction was also calculated. The extracts were concentrated under a stream of nitrogen to dryness. The residues were reconstituted with 1.0 ml of a solution of 70% of HPLC mobile phase A and 30% of B, vortexed, and centrifuged at 2000g for 5 min, and 50 μl of the supernatant was used for HPLC analysis.

**Metabolite Profiles, Identification, and Quantification.** Metabolite profiles. Metabolites in plasma, bile, urine, and fecal extract samples were analyzed using a Shimadzu LC-10AT system equipped with a photodiode array UV detector (Shimadzu, Kyoto, Japan). Samples (urine, bile, and extracts of plasma and feces) were injected onto a 4.6×150 mm YMC ODS AQ 5-μm column equipped with a guard column. The mobile phase consisted of two solvents: solvent A, 0.06% TFA in water; and solvent B, 0.06% TFA in acetonitrile. The gradient used was as follows: solvent B started at 5%, then linearly increased to 25% at 5 min, to 40% at 20 min, to 53% at 60 min, to 60% at 63 min, and to 90% at 65 min, was held at 90% for 7 min, and then decreased to 5% at 75 min. HPLC effluent (1 ml/min) was collected into plates (Deepwell LumaPlate-96; PerkinElmer Life and Analytical Sciences) at 0.26-min intervals for 75 min after injection with a model 202 fraction collector (Gilson, Inc., Middleton, WI). The plates were dried with a Speed-Vac (Savant; Thermo Fisher Scientific, Waltham, MA) and counted for 10 min/well with a TopCount analyzer (PerkinElmer Life and Analytical Sciences) to quantify radioactivity.

Biotransformation profiles were prepared by plotting the resulting net counts per minute values versus time after injection. Radiochromatograms were reconstructed from the TopCount data using Microsoft Excel software.

**Metabolite identification and quantification.** The pooled plasma, bile, urine, and fecal samples were analyzed by LC-MS using Finnigan LCQ and LTQ ion trap mass spectrometers (Thermo Fisher Scientific). The samples were analyzed by positive mode electrospray ionization. The HPLC system was a Shimadzu Class VP system equipped with two pumps, an autoinjector, and a diode array detector. The HPLC separation of the samples was performed using a 2.0 × 150 mm YMC ODS AQ S-3 120-Å column. The mobile phase flow rate was 0.28 ml/min. The gradient used was as the same as that described above. The HPLC effluent was directed to the mass spectrometer through a divert valve set to divert the flow to waste from 0 to 5 min. The capillary temperature was set at 210°C. The nitrogen gas flow rate, spray current, and voltages were adjusted to give maximum sensitivity for peliglitazar. The metabolites were quantified on the basis of the percentage of the total radioactivity of each peak observed in the entire HPLC radiochromatogram.

**Results**

**Excretion of Radioactive Dose.** After oral administration of [14C]peliglitazar, radioactivity was excreted predominantly in bile and feces. The recovery values of radioactivity from urine, bile, and feces after oral administration of [14C]peliglitazar to human subjects are presented in Table 1. Overall, the average recovery of total radioactivity was more than 95% for group 1 and 58% for group 2. The reason for the low recovery of radioactive dose in group 2 is unknown. Fecal excretion (over 240 h) accounted for approximately 94% (group 1) and 32% (group 2) of the radioactive dose. In addition, approximately 25% of the radioactive dose was recovered in bile collected from group 2 subjects. The majority of bile radioactivity was found in the 6- to 8-h interval samples (approximately 22% of the radioactive dose) probably due to gallbladder emptying stimulated by administration of cholecystokinin at 7 h postdose. Approximately 1.5% of the radioactive dose was found in the urine for both groups.

**Pharmacokinetic Parameters of Total Radioactivity.** After oral administration, the C_max of total radioactivity was determined at 1.1 h postdose in both groups. The mean half-life of radioactivity was approximately 3.5 h for both groups (Table 2). The mean AUC_{0–12 h} value from subjects with bile collection was similar to that from subjects without bile collection. Overall, the exposures of total radioactivity in group 2 subjects were similar to those in the group 1 subjects (Table 2).

**Metabolic Profiles of [14C]Peliglitazar.** Plasma. The recovery of radioactivity after extraction from plasma samples averaged 104.5% for group 1 and 96% for group 2. Figure 2 shows the metabolic profiles of plasma samples at 1, 4, 12, and 24 h (group 1). The parent compound and its glucuronide conjugate (M14) were the major components, representing 65 to 100% of the plasma radioactivity. In the 1-h sample, visible metabolites included glucuronide-conjugated metabolites M14, M15, M15a, and M17 and oxidative metabolite M3. Except for M14, other metabolites were relatively minor compared with the parent drug. The metabolic profiles in plasma from group 2 were qualitatively similar to those from group 1 (profiles not shown). The relative distribution of each metabolite in pooled plasma samples is listed in Table 3.

**Bile.** The metabolic profiles of pooled bile samples (grand pool, 3–4, 4–6, and 6–8 h) are shown in Fig. 3. The metabolic profiles of bile samples were qualitatively similar between the interval pooled sample and individual pooled sample (profiles not shown) as well as the grand pooled sample. The major bile metabolites in the grand pooled bile sample included the acyl glucuronide of peliglitazar (20% of the dose, three isomers M14, M14a, and M14b), glucuronides of hydroxy peliglitazar (1.5% of the dose, three isomers M15, M15a, and M15b), and glucuronides of O-demethyl peliglitazar (M17, 1.8% of the dose). The parent drug was only a minor component in bile and accounted for 0.46% of the dose. Other minor metabolites detected in bile were oxidative metabolites M1, M2, M3, M4, M5, M6, M7, and M8. The distribution of each radioactive metabolite in bile is listed in Table 4.

**Urine.** The relative distribution of the radioactive metabolites in urine is listed in Table 4. Minor metabolites in urine of both groups

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Recovery of Radioactivity</th>
<th>Subject No.</th>
<th>Recovery of Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
<td>Feces</td>
<td>Total</td>
</tr>
<tr>
<td>0002</td>
<td>1.67</td>
<td>101.1</td>
<td>102.8</td>
</tr>
<tr>
<td>0004</td>
<td>1.08</td>
<td>91.7</td>
<td>92.8</td>
</tr>
<tr>
<td>0006</td>
<td>0.73</td>
<td>98.8</td>
<td>99.5</td>
</tr>
<tr>
<td>0007</td>
<td>0.99</td>
<td>88.9</td>
<td>89.9</td>
</tr>
<tr>
<td>0009</td>
<td>2.81</td>
<td>88.3</td>
<td>91.6</td>
</tr>
<tr>
<td>0010</td>
<td>1.55</td>
<td>93.3</td>
<td>94.9</td>
</tr>
</tbody>
</table>
were the acyl glucuronide of peliglitazar (M14) and oxidative metabolites M2 and M3. Parent compound in urine was also a minor component.

**Feces.** The extraction recovery of radioactivity in fecal samples averaged 104%. The metabolic profiles of feces were qualitatively similar between groups (Fig. 4). The relative distribution of the radioactive metabolites in feces is listed in Table 4. Unchanged drug in feces accounted for approximately 47% of the dose in group 1. Major metabolites in feces of group 1 subjects were M1, M3, M4, M7, and M10. The results showed that peliglitazar was extensively metabolized by oxidative pathways in humans.

**Identification of Metabolites.** The structures of metabolites were elucidated by LC-MS and LC-MS/MS analysis, and chromatographic and mass spectral comparisons with the synthesized standards. Table 5 shows the structures of proposed peliglitazar metabolites. The parent compound (P), peliglitazar, showed a molecular ion \([M + H]^+\) at \(m/z\) 531 and major fragment ions at \(m/z\) 306 and 186 in LC-MS/MS analysis.

M1 showed a molecular ion \([M + H]^+\) at \(m/z\) 517 (531 - 14) and major fragment ions at \(m/z\) 306 and 186 in LC-MS/MS analysis. M1 was assigned as O-demethyl peliglitazar based on LC-MS/MS analysis and HPLC retention time comparison with a synthetic standard.

M2 showed a molecular ion \([M + H]^+\) at \(m/z\) 218 and major fragment ions at \(m/z\) 172. M2 was assigned as 5-methyl-2-
TABLE 3
Plasma radioactivity concentration and relative distribution of radioactive metabolites in pooled human plasma samples at 1, 4, 12, and 24 h after a single oral administration of 10 mg of $[^{14}C]$peliglitazar

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>1 h Group 1</th>
<th>1 h Group 2</th>
<th>4 h Group 1</th>
<th>4 h Group 2</th>
<th>12 h Group 1</th>
<th>12 h Group 2</th>
<th>24 h Group 1</th>
<th>24 h Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>M3</td>
<td>3.8</td>
<td>3.5</td>
<td>N.A.</td>
<td>3.8</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>M14</td>
<td>47.3</td>
<td>26.0</td>
<td>55.6</td>
<td>47.9</td>
<td>56.9</td>
<td>52.1</td>
<td>100</td>
<td>N.A.</td>
</tr>
<tr>
<td>M15</td>
<td>2.8</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>M15a</td>
<td>2.5</td>
<td>1.1</td>
<td>2.1</td>
<td>5.2</td>
<td>N.A.</td>
<td>10.4</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>M17</td>
<td>2.8</td>
<td>0.9</td>
<td>13.8</td>
<td>6.6</td>
<td>29.4</td>
<td>25.0</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Parent</td>
<td>38.2</td>
<td>67.2</td>
<td>28.6</td>
<td>34.3</td>
<td>13.7</td>
<td>12.5</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Total</td>
<td>97.4</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

N.A., not analyzed by LC-MS; negligible amounts of radioactivity.

M3 was assigned as O-dealkyl peliglitazar by comparing the HPLC retention time to the synthetic metabolite standard.

Trace indicates trace amounts by radioactivity and detectable by LC-MS.

FIG. 3. Metabolite profiles of the pooled bile samples of group 2 after single oral administration of 10 mg of $[^{14}C]$peliglitazar to humans. 1, 2, 3,…, and P represent M1, M2, M3,…, and parent compound.
phenytoxazyl-4-acetic acid based on LC-MS/MS analysis and HPLC retention time comparison with a synthetic standard. M3 was a minor metabolite in human samples and its molecular ion [M + H]^+ and fragment ions were not obtained under the study conditions. M3 was assigned as O-dealkyl peliglitazar based on HPLC retention time comparison with a synthetic standard.

M4, M5, and M6 had the same mass spectral fragmentation pattern and showed a molecular ion [M + H]^+ at m/z 547 (531 + 16), consistent with a hydroxylation. The major fragment ions were m/z 529 (547 – 18), 322 (306 + 16), 304 (322–18), and 202 (186 + 16) in LC-MS/MS analysis. Based on LC-MS/MS analysis and comparison with the synthetic standards, M4, M5, and M6 were assigned as hydroxy metabolites of peliglitazar.

M7 had a molecular ion [M + H]^+ at m/z 533 (531 + 16 – 14), consistent with hydroxylation and demethylation. The fragment ions were m/z 515 (533 – 18), 503, 322 (306 + 16), 304 (322 – 18), and 202 (186 + 16). Based on LC-MS/MS analysis and comparison with the synthetic standards, M7 was assigned as 12-hydroxy O-demethyl peliglitazar.

M8 had a molecular ion [M + H]^+ at m/z 533 (531 + 16 – 14), consistent with hydroxylation and demethylation. The fragment ions were m/z 515 (533 – 18), 479, 322 (306 + 16), 304 (322 – 18), and 202 (186 + 16). M8 was proposed as 9-hydroxy O-demethyl peliglitazar.

M10 yielded an ammonium adduct molecular ion [M + NH_4]^+ at m/z 538 in the positive ion mode and the major fragment ions were m/z 296, 176, and 105. It was consistent with a ring-opened metabolite of peliglitazar (mol. wt. = 520).

M14 was a major metabolite in human bile and plasma. This metabolite had a molecular ion [M + H]^+ at m/z 707 (531 + 176), consistent with a glucuronide of parent drug. The fragment ions were m/z 689 (707 – 18), 531(707 – 176), and 306 (Fig. 5). Based on LC-MS/MS analysis and comparison with the synthetic standard of β-acyl glucuronide of peliglitazar, M14 was assigned as the acyl glucuronide of peliglitazar. M14a and M14b showed a molecular ion [M + H]^+ at m/z 707 (531 + 176) and fragment ions at m/z 689 (707 – 18), 531 (707 – 176), and 306 (Fig. 5), also consistent with glucuronide conjugates of peliglitazar and were assigned as acyl migration isomers of M14.

M15 showed the same molecular ion [M + H]^+ at m/z 723 (531 + 16 + 176), consistent with a glucuronide of hydroxylated parent drug. The major fragment ions were m/z 705 (723 – 18), 687(677 – 18), 547 (723 – 176), 529 (547 – 18), 498 (322 + 176), 378 (186 + 16 + 176), 322 (306 + 16), and 304 (322 – 18). This was consistent with a structure of an ether glucuronide of hydroxy peliglitazar.

M15a showed the same molecular ion [M + H]^+ at m/z 723 (531 + 16 + 176) and major fragment ions were m/z 705 (723 – 18), 687(677 – 18), 547 (723 – 176), 529 (547 – 18), 498 (322 + 176), 378 (186 + 16 + 176), 322 (306 + 16), and 304 (322 – 18).
<table>
<thead>
<tr>
<th>Peak</th>
<th>RT (min)</th>
<th>Proposed Structure</th>
<th>Descriptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
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<td><img src="image" alt="Structure P" /></td>
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</tr>
<tr>
<td>M1</td>
<td>48.4</td>
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<td>21</td>
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</tr>
<tr>
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<td>39.5</td>
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</tr>
<tr>
<td>M6</td>
<td>46.8</td>
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<tr>
<td>M7</td>
<td>27.6</td>
<td><img src="image" alt="Structure M7" /></td>
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</tr>
<tr>
<td>M8</td>
<td>32.1</td>
<td><img src="image" alt="Structure M8" /></td>
<td>9-Hydroxy O-demethyl peliglitazar</td>
</tr>
</tbody>
</table>
M15a was also assigned as an ether glucuronide of hydroxy peliglitazar. M15b showed a molecular ion \([M+H]^+\) at \(m/z\) 723 (531 + 16), 547 (723 – 18), 547 (723 – 16), 322 (306 + 16), and 304 (322 – 18). The lack of fragment ions of \(m/z\) 498 and \(m/z\) 378 supported a structure of the acyl glucuronide of hydroxy peliglitazar.

M17 showed a molecular ion \([M+H]^+\) at \(m/z\) 693 (531 – 14 + 16), consistent with a glucuronide of demethyl parent drug. The major fragment ions were \(m/z\) 675 (693 – 18), 517 (693 – 16), and 306. M17 was assigned as an acyl glucuronide of \(O\)-demethyl peliglitazar, although the possibility of it being an ether glucuronide cannot be ruled out.

In summary, 16 metabolites of peliglitazar were identified in human samples, including a number of glucuronic acid conjugates of peliglitazar and its oxidative metabolites (in bile, plasma, and urine). The proposed metabolic pathways of peliglitazar in humans are shown in Fig. 6.

**Discussion**

The purpose of this study was to investigate the mass balance, disposition, and metabolism of dual labeled \([^{14}C]\)peliglitazar in healthy male human subjects after a single 10-mg oral dose with and without bile collection (groups 1 and 2). Because an \(O\)-dealkylation reaction was observed in studies with a structurally related analog, muraglitazar, that split the molecule into two large fragments and the \(O\)-dealkylation reaction was observed in incubations of peliglitazar in human hepatocytes (data not shown), dual labeled \([^{14}C]\)peliglitazar was used to ensure that the complete disposition profile of peliglitazar was captured.

The recovery of radioactivity averaged 94% of dose in feces for group 1 subjects. Group 2 showed a recovery value (58%) significantly lower than that of group 1. The pharmacokinetic parameters of radioactivity in plasma were similar between two groups (Table 2), indicating that dose administration was not an issue. The low recovery in group 2 could be the result of incomplete collection or homogenization of the fecal samples or a partial loss of the radioactivity in the bile collection tubing. The exact reason for the low recovery remains unclear.

In group 2 subjects, approximately 24% of the radioactivity was recovered in the bile during the 3 to 8 h collection period and 32% of the radioactivity was recovered from urine. The results indicated that biliary excretion was the major elimination route for peliglitazar and its metabolites in humans.
The $C_{\text{max}}$ of total radioactivity was reached at 1.1 h postdose, indicating rapid absorption of peliglitazar. The metabolic profiles of the 1-h plasma sample showed that peliglitazar (38–67%) and an acyl glucuronide of peliglitazar (M14, 26–47%) were the major drug-related components. No other metabolites represented more than 5% of the total plasma radioactivity. By 24 h postdose, the plasma profiles showed that the concentrations of radioactivity had declined significantly, and M14 was the only visible radioactive peak.

Although the bile collection was limited to a relatively short period of time after dose administration, the recovery in the 3 to 8 h bile accounted for approximately 24% of the radioactive dose and provided important additional information on the metabolic pathways and the excretion route of peliglitazar. The major metabolites in feces were characterized by LC-MS/MS as oxidative metabolites. Bile collected from 3 to 8 h in group 2 subjects contained 24% of the radioactive dose, and major metabolites in bile were glucuronide conjugates of peliglitazar and its metabolites, accounting for approximately 23% of the radioactive dose. These results indicated that 1) glucuronide conjugation was an important metabolic pathway for peliglitazar and 2) the glucuronide conjugates were excreted into bile as the major elimination route for peliglitazar in humans. Conjugation seems to be the predominant clearance pathway, but hydrolysis sets up a situation for enterohepatic recirculation of peliglitazar and metabolites. Although reabsorption of these compounds undoubtedly occurs to some extent, the low levels of oxidative metabolites in circulation argue against significant recirculation of peliglitazar metabolites. Peliglitazar in feces could be from nonabsorbed drug and hydrolysis of the glucuronide of metabolites that were formed in the liver, excreted into bile, and hydrolyzed in the intestinal tract.

The formation of an acyl glucuronide was the predominant metabolic route of peliglitazar in humans. The isomers detected were formed by acyl migration. Muraglitazar, also a dual $\alpha$/$\gamma$ PPAR activator with a molecular structure similar to that of peliglitazar, was extensively metabolized in humans, and the major metabolite was also an acyl glucuronide eliminated in the bile (Wang et al., 2006; Zhang et al., 2007a,b). However, the major circulating component in humans was parent compound after oral administration of muraglitazar with very little glucuronide detected (Wang et al., 2006). The stability of peliglitazar acyl glucuronide was a concern because acyl glucuronide-mediated toxicity has been reported in the literature (Boelsterli, 2003; Sawamura et al., 2010). In the current study, the recovery of radioactivity from plasma extraction averaged 104.5% in group 1 and 96% in group 2. This result indicated that there was very little plasma protein covalent binding, and the acyl glucuronide of peliglitazar was relatively stable in samples acidified immediately after collection and stored at $-20^\circ\text{C}$. Other experiments to look at the stability of peliglitazar glucuronide were also performed, and the results are reported separately (Zhang et al., 2010).

In summary, the present study demonstrates that [$^{14}$C]peliglitazar was rapidly absorbed after oral administration. Most of the radioactive dose was recovered in feces or bile. [$^{14}$C]Peliglitazar was extensively metabolized in humans. The parent drug and a glucuronide conjugate...
were the predominant circulating components. Biliary elimination of glucuronide conjugates was a major clearance pathway for peliglitazar; however, the glucuronides were not present in fecal samples, leading to the conclusion that they were hydrolyzed in the intestines before excretion. In conclusion, very different metabolic profiles in circulation were found for muraglitazar and peliglitazar, two close structural analogs, although the overall dispositional profiles were similar between muraglitazar and peliglitazar.

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Authorship Contributions

Participated in research design: Wang, Humphreys, and Zhang.
Conducted experiments: Wang.
Contributed new reagents or analytic tools: Wang, Chen, Bonacorsi, and Cheng.
Performed data analysis: Wang, Munsick, Humphreys, and Zhang.
Wrote or contributed to the writing of the manuscript: Wang, Munsick, Chen, Bonacorsi, Cheng, Humphreys, and Zhang.

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