GLUCURONIDATION AS A MAJOR METABOLIC CLEARANCE PATHWAY OF 
14C-LABELED MURAGLITAZAR IN HUMANS: METABOLIC PROFILES IN SUBJECTS 
WITH OR WITHOUT BILE COLLECTION

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ABSTRACT:
The metabolism and disposition of 14C-labeled muraglitazar (Paragluva), a novel dual α/γ peroxisome proliferator-activated receptor activator, was investigated in eight healthy male subjects with and without bile collection (groups 1 and 2) after a single 20-mg oral dose. Bile samples were collected for 3 to 8 h after dosing from group 2 subjects in addition to the urine and feces collection. In plasma, the parent compound was the major component, and circulating metabolites, including several glucuronide conjugates, were minor components at all time points. The exposure to parent drug (Cmax and area under the plasma concentration versus time curve) in subjects with bile collection was generally lower than that in subjects without bile collection. The major portion of the radioactive dose was recovered in feces (91% for group 1 and 51% for group 2). In addition, 40% of the dose was recovered in the bile from group 2 subjects. In this 3- to 8-h bile, the glucuronide of muraglitazar (M13, 15% of dose) and the glucuronides of its oxidative metabolites (M17a,b,c, M18a,b,c, and M20, together, 16% of dose) accounted for approximately 80% of the biliary radioactivity; muraglitazar and its O-demethylated metabolite (M15) each accounted for approximately 4% of the dose. In contrast, fecal samples only contained muraglitazar and its oxidative metabolites, suggesting hydrolysis of biliary glucuronides in the intestine before fecal excretion. Thus, the subjects with and without bile collection showed different metabolic profiles of muraglitazar after oral administration, and glucuronidation was not observed as a major pathway of metabolic clearance from subjects with the conventional urine and fecal collection, but was found as a major elimination pathway from subjects with bile collection.

Peroxisome proliferator-activated receptors (PPARs) are a set of nuclear hormone receptors (comprising the α, γ, and δ subtypes) which act as transcription factors in the regulation of multiple genes involved in such diverse disease areas as type 2 diabetes, dyslipidemia, obesity, inflammation, cancer, and osteoporosis (Torró et al., 2001; Taskinen, 2003; Yajima et al., 2004). The two most intensively investigated subtypes have been PPARα (primarily expressed in the liver and which plays a critical role in lipid metabolism) and PPARγ (predominantly expressed in adipose tissue and 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; Despres, 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; Cheng-Lai and Levine, 2000; Goldstein, 2000) and pioglitazone (Gilley and Dunn, 2000).

Muraglitazar (Paragluva), N-[4-methoxyphenoxy]carbonyl]-N-[4-[2-(5-methyl-2-phenyl-4-oxazolyl)ethoxy]phenyl][methyl]-glycine, is a novel dual α/γ PPAR activator, and the structure of muraglitazar is shown in Fig. 1. It has been shown that muraglitazar has both glucose- and lipid-lowering effects when tested in animal models of diabetes and dyslipidemia and in patients with diabetes (Devasthale et al., 2005; Mosqueda-Garcia et al., 2005). Muraglitazar is currently under development for the treatment of type 2 diabetes. From a human ADME study with urine and feces collection after a single 10-mg oral dose of [14C]muraglitazar, fecal excretion represented >90% of muraglitazar elimination, and only oxidative metabolites were identified in feces of human subjects (Zhang et al., 2006). However, in a disposition study of [14C]muraglitazar in bile duct-cannulated rats, biliary excretion represented >90% of elimination of muraglitazar, and glucuronide conjugates were the major metabolites in the rat bile (D. Zhang, personal communication). We suspected that glucuronidation might also be the major metabolic pathway of muraglitazar in humans, which was not investigated in the previous human ADME study (Zhang et al., 2006). To further explore the metabolism, disposition, and pharmacokinetics of [14C]muraglitazar, especially the role of glucuronidation and biliary elimination, a single 20-mg oral dose was administered to groups of subjects with or without bile collection.

ABBREVIATIONS: PPAR, peroxisome proliferator-activated receptor; ADME, absorption, distribution, metabolism, and excretion; AUC, area under the plasma concentration versus time curve; HPLC, high pressure liquid chromatography; EtO, diethyl ether; EtOAc, ethyl acetate; THF, tetrahydrofuran; MeOH, methanol; LC/MS, liquid chromatography/mass spectrometry; LC/MS/MS, LC/tandem mass spectrometry; LLOQ, lower limit of quantitation; QC, quality control; TFA, trifluoroacetic acid.
Sixteen oxidative human metabolites of \([^{14}C]\)muraglitazar have been identified and presented previously (Zhang et al., 2006). These metabolites were also found in the current study, and they included hydroxy muraglitazar (M8a, M10, M11, and M14), O-demethyl muraglitazar (M15), O-demethyl hydroxy muraglitazar (M2, M5, M6, and M7), oxazole ring-opening metabolites (M9 and M16), O-dealkyl muraglitazar (M1), dihydroxy muraglitazar (M3, M4, and M8), and muraglitazar 12-carboxylic acid (M12). Their structures and the proposed pathways for oxidation of muraglitazar are shown in Fig. 1.

**Materials and Methods**

**Radiolabeled Drug and Chemicals.** Muraglitazar was synthesized in the Discovery Chemistry Department at Bristol-Myers Squibb (Princeton, NJ). \([^{14}C]\)Muraglitazar was synthesized in the Radiochemistry/Chemical Synthesis Department, Bristol-Myers Squibb. The specific activity of \([^{14}C]\)muraglitazar was 5.13 \(\mu\)Ci/mg (99.6% radiochemical purity). 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.
acetone (10 ml) and water (40 ml) was added sodium bicarbonate (NaHCO₃, 5.5 g, 39 mmol). The resulting solution was stirred for 30 min at room temperature, after which benzoyl chloride (4.25 g, 33 mmol) was added dropwise, followed by potassium carbonate (K₂CO₃, 4.8 g, 34 mmol). The mixture was stirred for 1 h at room temperature and then was partitioned between water (20 ml) and diethyl ether (Et₂O). The aqueous layer was acidified with aqueous 2 N hydrochloric acid (HCl) to give a white precipitate. The precipitate was filtered off and dried overnight in vacuo to give the desired product. The resulting solution was stirred at room temperature overnight and then was filtered. The filtrate was concentrated in vacuo to give the desired product.

To a room temperature solution of intermediate 1 (5.0 g, 20 mmol) in pyridine (20 ml) was added the 2-benzoyloxycetonic anhydride prepared above. The reaction was stirred at 80°C for 4 h and then was cooled to room temperature and concentrated in vacuo. The residue was partitioned between aqueous 1 N HCl (30 ml) and Et₂O. The aqueous layer was then washed with aqueous 2 N HCl and further extracted with Et₂O. The combined organic extracts were dried over magnesium sulfate (MgSO₄) and concentrated in vacuo. The resulting solution of the crude 2-benzyloxyacetic anhydride was added dropwise to a solution of dicyclohexyl carbodiimide (7.45 g, 36 mmol) in methylene chloride (CH₂Cl₂, 20 ml) at room temperature. The reaction was stirred at room temperature for 4 h, then was partitioned between CH₂Cl₂ and aqueous NaHCO₃. The organic phase was washed with aqueous 1 N HCl and further extracted with Et₂O. The combined organic extracts were dried over magnesium sulfate (MgSO₄) and concentrated in vacuo. The residue was chromatographed (silica gel, hexane/EtOAc 85:15, v/v) to give the chloromethyloxazole intermediate as a pale yellow oil.

A solution of 2-benzyloxyacetic anhydride (5.0 g, 30 mmol) in methylene chloride (CH₂Cl₂, 20 ml) was added dicyclohexyl carbodiimide (DCC, 5 ml, 0.67 mmol). The reaction was stirred at 80°C for 4 h and then was cooled to room temperature and concentrated in vacuo. The residue was partitioned between CH₂Cl₂ and aqueous NaHCO₃. The organic phase was washed with aqueous 1 N HCl and further extracted with Et₂O. The combined organic extracts were dried over magnesium sulfate (MgSO₄) and concentrated in vacuo. The residue was chromatographed (silica gel, hexane/EtOAc 85:15, v/v) to give the chloromethyloxazole intermediate as a pale yellow oil.

NMR Experiments. NMR analyses of M10 and M11 were performed on a Bruker 400 MHz NMR spectrometer (heteronuclear multiple bond correlation, long range carbon-proton correlation) and edited distortionless enhancement by polarization transfer experiments were performed. All chemical shifts are reported in part per million (ppm) relative to tetramethylsilane in chloroform (CDCl₃).

Synthesis of Metabolite M10. The synthetic scheme of metabolite M10 is shown in Fig. 2A.

To a mixture of 5 g of l-aspartic acid β-methyl ester (5.0 g, 27 mmol) in methanesulfonyl chloride (CH₂Cl₂, 20 ml) was added dicyclohexyl carbodiimide (DCC, 5 ml, 0.67 mmol). The reaction was stirred at room temperature for 4 h, then was partitioned between CH₂Cl₂ and aqueous NaHCO₃. The organic phase
was dried (MgSO₄) and concentrated in vacuo. The residue was chromato-
graphed (silica gel, stepwise gradient from 85:15 to 60:40 hexane/EtOAc, v/v)
to give the tert-butyldimethylsilyl ether-mesyate intermediate 5 (170 mg, 60% for
3 steps) as a pale yellow oil. ¹H NMR (400 MHz, CDCl₃); 0.07 (s, 6 H),
0.85 (s, 9H), 2.87 (s, 3H), 2.96 (t, J = 6.5 Hz, 2H), 4.47 (t, J = 6.5 Hz, 2H),
4.65 (s, 2H), 7.36 (m, 3H), 7.91 (m, 2H). LC/MS [M + H⁺]: 412.
A mixture of phenol intermediate 6 (170 mg, 0.48 mmol; Devashahle et al.,
2005), mesylate intermediate 7 (100 mg, 0.24 mmol), and K₂CO₃ (40 mg, 0.29
mmol) in acetonitrile (5 ml) was stirred at 90°C for 36 h, and then was cooled to
room temperature. The mixture was concentrated in vacuo and the residue
was partitioned between saturated aqueous NaHCO₃ and EtOAc. The organic phase
was dried (MgSO₄) and concentrated in vacuo to give the alkyloxazole methyl-
oxide intermediate as a yellow oil.
To a solution of the crude protected hydroxymethyloxazole from the pre-
vious step in THF (10 ml) was added tetrabutylammonium fluoride (n-Bu₄NF,
1 ml of a 1 M solution in THF, 1 mmol) at room temperature. The reaction
was stirred at room temperature for 1 h and then was concentrated in vacuo to
give the crude alcohol.
A solution of the crude alcohol from the previous reaction and aqueous
sodium hydroxide (NaOH, 1 ml of a 1 M solution) in methanol (MeOH, 5 ml)
was stirred at room temperature for 4 h, and then was acidified to pH 3 with
aqueous 1 N HCl. Volatiles were removed in vacuo and the residue was
partitioned between EtOAc and saturated sodium chloride. The organic phase
was concentrated in vacuo, and the residue was purified by preparative HPLC
(Phenomenex Luna C18 column, 21.2 × 100 mm, 5 µ; detection at 220 nm; flow rate
= 25 ml/min; continuous gradient from 50% solvent A to 100% solvent B over 8 min). The
HPLC solvent was removed by rotary evaporation under vacuum and lyoph-
ilization to give metabolite M10 (23 mg; 9%) as a white powder. ¹H NMR (400
MHz, CDCl₃): 8.01 (m, 2H), 7.45 (m, 3H), 7.25 (m, 2H), 7.03 (m, 2H), 6.85
(m, 4H), 4.72 (d, 2H), 4.03 (2s, 2H), 3.78 (s, 3H), 3.09 (J = 5.9 Hz, 2H).
LC/MS [M + H⁺]: 533.
Synthesis of Metabolite M11. The synthetic scheme of the metabolite M11 is
outlined in Fig. 2B.
To a mixture of L-aspartic acid β-methyl ester hydrochloride (5.0 g, 27
mmol) in acetonitrile (10 ml) and H₂O (40 ml) was added NaHCO₃ (5.5 g). The solution was stirred at room temperature for 30 min, after which 4-benzoyloxy-
benzoyl chloride (4.25 g, 33 mmol) and K₂CO₃ (4.75 g, 34 mmol) were
successively added. The reaction mixture was stirred for 1 h at room temper-
ature and then was partitioned between CH₂Cl₂ (30 ml) and saturated aqueous NaHCO₃. The organic phase
was dried (MgSO₄) and concentrated in vacuo to give the oxazole mesylate
intermediate as a yellow oil.
A solution of the crude oxazole-ester from the previous reaction and
aqueous NaOH (1 ml of a 1 M solution) in MeOH (10 ml) was stirred at room
temperature for 18 h, and then was acidified with aqueous 1 N HCl. Volatiles
were removed in vacuo, and the residue was partitioned between EtOAc and
aqueous 1 N HCl. The organic phase was concentrated in vacuo and the residue
was purified by preparative HPLC (Phenomenex Luna C18 column, 21.2 ×
100 mm, 5 µ; detection at 220 nm; flow rate = 25 ml/min; continuous gradient from
50% solvent A to 100% solvent B over 8 min). The HPLC solvent was removed by rotary evaporation under vacuum and lyophilization to
give the carbamate-acid as a solid.
To a solution of the O-benzylphenyl carbamate acid from the previous step in
MeOH (10 ml) was added 10% palladium on carbon catalyst (100 mg), and
the reaction was stirred under an atmosphere of hydrogen at room temperature
for 30 min, after which the catalyst was filtered off. The filtrate was concen-
trated in vacuo and the residue was purified by preparative HPLC (Phenome-
nox Luna C18 column, 21.2 × 100 mm, 5 µ; detection at 220 nm; flow rate
= 25 ml/min; continuous gradient from 75% solvent A to 100% solvent B over 8 min). The HPLC solvent was removed by rotary evaporation under vacuum and lyophilization to
give the carbamate-acid as a solid.
Human Studies. The study was performed in accordance with the follow-
ing codes and guidelines: Title 21, Part 56 CFR (Institutional Review Board
Approval); Title 21, Part 50 CFR (Protection of Human Subjects); the princi-
ples of the Declaration of Helsinki and its amendments; and Good Clinical
Practice. After being advised of the nature and risks associated with the study,
all subjects were required to give informed and written consent before partic-
ipation in the study. All subjects were in good health as determined by medical
history, physical examination, and clinical laboratory tests conducted before
the study based on inclusion and exclusion criteria.
A total of eight healthy male subjects, aged 18 to 45 years, were assigned to
two groups (groups 1 and 2, four subjects each group). After at least an 8-h
overnight fast, each subject (groups 1 and 2) received a single dose of 20 mg of
[¹⁴C]mugraflitazar containing 102.6 µCi of radioactivity as an oral solution
in PEG-400 (polyethylene glycol 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 ≥5% of administered radioactivity.
Collection of Blood, Urine, and Feces. Blood samples for serial pharma-
cokinetic analysis were drawn (5 ml each) before dosing and postdose at 0.25, 0.5, 1, 1.5, 2, 4, 8, 12, 24, 48, 72, 96, and 120 h. 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 containing K₃EDTA as the anticoagulant. Plasma was prepared by
centrifugation at approximately 1000g for 15 min at 4°C. Acetic acid was added
to plasma to a final concentration of 5% (v/v, 0.83 M). Immediately after
processing, all plasma samples were frozen and stored at −20°C.
Bile samples were collected from group 2 subjects during 3- to 4-, 4- to 6-, and 6- to 8-h periods postdose using duodenal intubation and aspiration methods described previously (Strasberg et al., 1990; Choudhuri et al., 1993). One hour after drug administration, the terminal end of an oral-gastro-duodenal tube was positioned at the vertical limb of the duodenal loop, near the ampulla of Vater (confirmed via fluoroscopy). An intravenous dose of cholecystokinin was given to stimulate gallbladder contraction at 7 h postdose. Acetic acid was added after each collection to a final concentration of 5% (v/v, 0.83 M). The bile samples were immediately frozen and stored at −70°C. Urine samples were collected over 24-h intervals throughout the study (0–240 h) from all subjects. The urine was collected in chilled urine collection jugs containing 50 ml of acetic acid, and the jugs were stored refrigerated during the collection periods. At the end of each collection interval, the total urine sample was mixed thoroughly and total volume was recorded to the nearest milliliter. Acetic acid was added after each collection to a final concentration of 5% (v/v). All urine samples were frozen and stored at −20°C.

Feces samples were collected over 24-h intervals throughout the study (0–240 h) from all subjects. All feces samples were frozen and stored at −20°C. Fecal homogenate was prepared as follows. The fecal samples were thawed and the total weight of each fecal sample was recorded before processing; a volume of 5% (v/v) acetic acid in 50% reagent ethanol was added to each sample to form an approximate 20% (w/w) feces/solvent homogenate; a volume of 5% (v/v) acetic acid in 50% reagent ethanol was added to each sample to form an approximate 20% (w/w) feces/solvent homogenate; finally, the total weight was recorded and each sample was homogenized by using a probe-type homogenizer. All fecal homogenates were stored at −20°C.

Measurement of Radioactivity. The levels of total radioactivity in plasma, urine, bile, and feces were determined by liquid scintillation counting after an aliquot of each sample was combusted. Sample combustion was performed by using a model A0387 sample oxidizer (PerkinElmer Life and Analytical Sciences, Boston, MA). The resulting 14CO2 was trapped with Carbo-Sorb E (PerkinElmer Life and Analytical Sciences), mixed with Permafluor E+ (PerkinElmer Life and Analytical Sciences) scintillation fluid, and the radioactivity was quantified using liquid scintillation counting. The combustion efficiency was determined before combustion of experimental samples using a commercial carbon-14 standard. Carbon-14-spiked scintillation fluid or fecal homogenate samples spiked with the same amount of radioactivity at three commercial carbon-14 standards. Carbon-14-spiked scintillation fluid or fecal homogenate samples spiked with the same amount of radioactivity were combusted and then counted. The average disintegrations per minute (dpm) recovered after combustion was compared in spiked scintillation fluid or fecal homogenate samples spiked with the same amount of radioactivity at three levels of radioactivity, corresponding to low, medium, and high standards, were combusted and then counted. The average disintegrations per minute (dpm) recovered after combustion was compared in spiked scintillation fluid or fecal homogenate samples spiked with the same amount of radioactivity at three levels of radioactivity, corresponding to low, medium, and high standards, were combusted and then counted. The average disintegrations per minute (dpm) recovered after combustion was compared in spiked scintillation fluid or fecal homogenate samples spiked with the same amount of radioactivity at three levels of radioactivity, corresponding to low, medium, and high standards, were combusted and then counted.

Quantification of Muraglitazar in Plasma. Plasma concentrations of unchanged muraglitazar were determined by a validated protein precipitation, liquid chromatography/tandem mass spectrometry (LC/MS/MS) method (Y.-J. Xue, J. Liu, J. Pursley, and S. Unger, manuscript submitted for publication). The internal standard was dissolved into 0.1% formic acid in acetonitrile, which also served as a protein precipitation reagent. Human plasma samples (0.1 ml) and the internal standard solution (0.3 ml) were added to a 96-well plate. The plate was vortexed for 1 min and centrifuged for 5 min, after which the supernatant samples were directly injected into the LC/MS/MS apparatus. Chromatographic separation was achieved isocratically on a Phenomenex Luna C18 column (2 × 50 mm, 5 μ). The mobile phase contained 20% of 1 mM formic acid in water and 80% of 1 mM formic acid in acetonitrile. Detection was by positive ion electrospray tandem mass spectrometry on a Sciex API 4000 (Applied Biosystems/MDS Sciex, Foster City, CA). The transition monitored was m/z 517 to m/z 186 for muraglitazar. The standard curve, which ranged from 1 to 1000 ng/ml, was fitted to a 1/x 2 weighted quadratic regression model. All plasma samples were analyzed within a total of four analytical runs. The LLOQ of muraglitazar concentration in plasma under these conditions was 1 ng/ml. QC samples were analyzed along with the study samples to assess the accuracy and precision of the assay. The acceptance criteria established for the analysis of muraglitazar in plasma specified that the predicted concentrations of at least three-fourths of the standards and two-thirds of the QC samples be within ±15% of their individual nominal concentration values (±20% for the lowest concentration standard). In addition, at least one QC sample at each concentration had to fall within ±15% of its individual nominal concentration value. Values for the between-run precision and the within-run precision for analytical quality control samples were no
greater than 0% and 11.1% coefficient of variation (CV), respectively, with deviations from the nominal concentrations of no more than ±5.2%.

**Pharmacokinetic Analysis.** The mean plasma concentration versus time data for the radioactivity and unchanged muraglitazar were analyzed by a noncompartmental method (Gibaldi and Perrier, 1982). The peak plasma concentration, $C_{\text{max}}$, and the time to reach peak concentration, $T_{\text{max}}$, 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 120 h. The first order rate constant of decline of radioactivity concentration and unchanged muraglitazar, expressed as equivalents of muraglitazar, in the terminal phase of each plasma concentration versus time profile, $K_{\text{t,1/2}}$, 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 $K_{\text{t,1/2}}$ was used to estimate the apparent terminal elimination half-life, $t_{1/2}$.

**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 methanol and 3 ml acetonitrile and mixed on a Vortex mixer. The mixtures were centrifuged at 2000 g for 10 min; then, 100 μl of the supernatant was used for the HPLC analysis.

A grand pooled bile sample 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. A portion (0.1 ml) of each pooled bile sample was diluted 10 times with the HPLC mobile phase A, and 150 μl of the diluted sample was used for the HPLC analysis.

Two grand pooled urine samples (0–240 h) from groups 1 and 2 were prepared separately by mixing 1% of urine by weight of each collection from all subjects in each group. The interval pooled bile samples (3–4 h, 4–6 h, and 6–8 h) were prepared by mixing 1% of bile by volume from each collection interval of all subjects. The individual pooled bile samples were prepared by mixing 3% of bile 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 stream of nitrogen. The residues were then reconstituted with 1.0 ml of a solution of 70% HPLC mobile phase A (0.06% TFA in water) and 30% mobile phase B (0.06% TFA in acetonitrile), vortexed, and centrifuged at 2000g for 10 min, and 150 μl of the supernatant was used for the HPLC analysis.

Two grand pooled fecal homogenate samples (0–240 h) from groups 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

**TABLE 2**

**Summary of pharmacokinetic parameters of total radioactivity and unchanged drug after a single oral dose of [14C]muraglitazar**

Values are means.

<table>
<thead>
<tr>
<th>Group</th>
<th>Radioactivity</th>
<th>Unchanged Muraglitazar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_{\text{max}}$ (CV%)</td>
<td>AUC(0–120 h) (CV%)</td>
</tr>
<tr>
<td>All subjects ($n = 8$)</td>
<td>2582 (21)</td>
<td>14224 (25)</td>
</tr>
<tr>
<td>Group 1 ($n = 4$)</td>
<td>3002 (14)</td>
<td>14966 (19)</td>
</tr>
<tr>
<td>Group 2 ($n = 4$)</td>
<td>2220 (16)</td>
<td>13482 (33)</td>
</tr>
</tbody>
</table>

* $P < 0.05$, t test, group 1 vs group 2.
(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 and then centrifuged at 2000 g 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 recovery of radioactivity averaged 86%. The extracts were concentrated under a stream of nitrogen to dryness. The residues were reconstituted with 1.0 ml of a solution of 70% HPLC mobile phase A and 30% B, vortexed, and centrifuged at 2000 g for 5 min; then, 50 µl of the supernatant was used for HPLC analysis.

**Metabolite Profiles, Identification, and Quantification.** Metabolites in plasma, bile, urine, and fecal extract samples were analyzed by using a Shimadzu LC-10AT system equipped with a photodiode array UV detector (Shimadzu Scientific Instruments, Kyoto, Japan). Samples (urine, bile, extracts of plasma and feces) were injected onto a 4.6 x 150 mm, YMC ODS AQ, 5-µm column equipped with a guard column. The mobile phase consisted of two solvents: A, 0.06% TFA in water; and B, 0.06% TFA in acetonitrile. The gradient employed 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, to 90% at 65 min, held at 90% for 7 min, and then decreased to 5% at 75 min. HPLC effluent (1 ml/min) was collected into plates of Deepwell LumaPlate-96 (PerkinElmer Life and Analytical Sciences) at 0.26-min intervals for 75 min after injection with a Gilson model 202 fraction collector (Gilson Medical Electronics, Middleton, WI). The plates were dried with a SpeedVac (Savant, Holbrook, NY) and counted for 10 min per well with a TopCount analyzer (PerkinElmer Life and Analytical Sciences) to quantify radioactivity. Biotransformation profiles were prepared by plotting the resulting net cpm values versus time after injection. Radiochromatograms were reconstructed from the TopCount data using Microsoft Excel software (Microsoft, Redmond, WA).

**TABLE 3**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feces</td>
<td>Urine</td>
</tr>
<tr>
<td>% of dose</td>
<td>% of dose</td>
<td>% of dose</td>
</tr>
<tr>
<td>M1</td>
<td>1.0</td>
<td>0.27</td>
</tr>
<tr>
<td>M2</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>M3</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>2.0</td>
<td>0.70</td>
</tr>
<tr>
<td>M5</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>M6</td>
<td>0.90</td>
<td>0.4</td>
</tr>
<tr>
<td>M7</td>
<td>1.0</td>
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</tr>
<tr>
<td>M8a</td>
<td>2.8</td>
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</tr>
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<td>M8b</td>
<td>3.3</td>
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</tr>
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</tr>
<tr>
<td>M13</td>
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</tr>
<tr>
<td>M14</td>
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<td>0.30</td>
</tr>
<tr>
<td>M15</td>
<td>3.4</td>
<td>4.2</td>
</tr>
<tr>
<td>M16</td>
<td>12</td>
<td>9.5</td>
</tr>
<tr>
<td>M17a</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>M17b</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>M17c</td>
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<td></td>
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<tr>
<td>M18a</td>
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<tr>
<td>M18b</td>
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<td>0.84</td>
</tr>
<tr>
<td>M18c</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>M20</td>
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<td>2.9</td>
</tr>
<tr>
<td>M21</td>
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<td>0.46</td>
</tr>
<tr>
<td>Parent</td>
<td>23</td>
<td>0.23</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>2.1</td>
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* M2, M5, M7, M6a, M8, M11, and M14 were also detected in bile by LC/MS but in trace amounts by radioactivity.

Fig. 6. Biotransformation profiles of pooled urine (A) and pooled fecal homogenates (B) after single oral administration of 20 mg of [14C]muraglitazar. 1, 2, 3,..., and P represent M1, M2, M3,..., and parent compound. The samples were analyzed by HPLC as described under Materials and Methods.

**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 (ThermoFinnigan, San Jose, CA). 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 x 150 mm, YMC ODS AQ, S-5 120-Å column. The mobile phase flow rate was 0.28 ml/min. The gradient used was the same as 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 a maximum sensitivity for muraglitazar. The metabolites were quantified based on 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]muraglitazar, radioactivity was excreted predominantly in bile and feces. The cumulative biliary, fecal, and urinary excretion of radioactivity and radioactivity recovery values from human subjects after oral administration of [14C]muraglitazar are presented in Table 1 and Fig. 3. Overall, the average recovery of total radioactivity (in urine, bile, and feces) was more than 93% for both groups. Fecal excretion accounted for about 91% (group 1) and 51% (group 2) of the radioactive dose during the 0- to 240-h collection period. In addition, approximately 40% 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 (about 35% of the radioactive dose), probably because of gallbladder emptying stimulated by administration of cholecystokinin at 7 h postdose. Approximately 2% (group 1) or 4% (group 2) of the radioactive dose was found in the urine. These results indicated that the fecal route via biliary excretion was the major elimination pathway for [14C]muraglitazar in humans.

Pharmacokinetic Parameters. The mean pharmacokinetic parameters for the total radioactivity and unchanged muraglitazar are presented in Table 2. The mean plasma concentration versus time profiles for the total radioactivity and unchanged muraglitazar after a single oral dose of [14C]muraglitazar are presented in Fig. 4. After oral administration, the \( C_{max} \) of the total radioactivity or unchanged drug was determined to be 1.5 h postdose in both groups.

The individual AUC_{0–120 h} values of muraglitazar were 13,765, 14,520, 12,700, and 10,440 ng · h/ml for group 1 subjects and 12,469, 10,438, 10,309, and 8,407 ng · h/ml for group 2 subjects. Three of the four AUC_{0–120 h} values from subjects with bile collection were lower than all four AUC_{0–120 h} values from subjects without bile collection. The largest AUC_{0–120 h} from the group 2 with bile collection was still lower than three of the four AUC_{0–120 h} values from subjects without bile collection. Overall, the exposures of total radioactivity and unchanged drug in group 2 subjects were 10% and 20% lower than those in the group 1 subjects (Table 2). The AUC_{0–120 h} of muraglitazar in plasma represented 77% of the AUC of the total radioactivity recovered for both groups, indicating that the major circulating radioactivity was from unchanged muraglitazar.

The different \( t_{1/2} \) values for unchanged muraglitazar and total radioactivity (20 versus 14.3 h) were probably due to their different lower limit of quantitation values. The time points used to calculate the apparent elimination \( t_{1/2} \) for radioactivity were within 48 h because of an LLOQ of 28.2 ng/ml, whereas the parent compound was detectable up to 120 h by LC/MS/MS analysis with an LLOQ of 1 ng/ml.

Metabolic Profiles of [14C]Muraglitazar. Plasma. Figure 5 shows the metabolic profiles in plasma extracts at 1, 4, 12, and 24 h after a single 20-mg oral dose of [14C]muraglitazar to humans (group 1). The parent compound was the major component, representing 70 to 100% of the plasma radioactivity. At 1 h, observed metabolites included oxidative metabolite M16 (1.1%), and glucuronide metabolites M13 (3.3%), M18a (trace amount), M18b (1.0%), M18c (0.7%), and M20 (1.1%). These metabolites were relatively minor, compared with the parent drug, and their identities were verified by LC/MS/MS analyses. The metabolic profiles in plasma at 1, 4, 12, and 24 h from group 2 subjects were qualitatively similar to those from group 1 subjects (profiles not shown).

Urine. The metabolic profiles of urine samples were qualitatively similar between group 1 and group 2 (Fig. 6A). The distribution of the radioactive metabolites in urine is listed in Table 3. The predominant radioactive metabolite in urine of both groups was the acyl glucuro-
Characterization of muraglitazar metabolites in humans after a single oral administration of [14C]muraglitazar

<table>
<thead>
<tr>
<th>Peak</th>
<th>RT (min)</th>
<th>Molecular Mass (Da)</th>
<th>Major Fragment Ions</th>
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<tr>
<td>P</td>
<td>61.8</td>
<td>516</td>
<td>292, 186</td>
<td>Muraglitazar</td>
</tr>
<tr>
<td>M1</td>
<td>18.1</td>
<td>331</td>
<td>226</td>
<td>0-Dealkyl muraglitazar</td>
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<td>M2</td>
<td>22.5</td>
<td>518</td>
<td>308, 242</td>
<td>O-Demethyl 8-hydroxy muraglitazar</td>
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<tr>
<td>M3</td>
<td>23.7</td>
<td>547</td>
<td>324, 187</td>
<td>8,12-Dihydroxy muraglitazar</td>
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<tr>
<td>M4</td>
<td>24.5</td>
<td>548</td>
<td>324, 306, 218</td>
<td>9,12-Dihydroxy muraglitazar</td>
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<tr>
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<td>25.0</td>
<td>518</td>
<td>398, 308, 290, 281</td>
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<td>M6</td>
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<td>M7</td>
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<td>352</td>
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<td>M8b</td>
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<td>Ether glucuronide of O-demethyl 9-hydroxy muraglitazar</td>
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<tr>
<td>M17b,c</td>
<td>22, 23.5</td>
<td>694</td>
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<td>M18a,c</td>
<td>25, 27</td>
<td>708</td>
<td>691, 533, 515, 508, 290, 202</td>
<td>Ether glucuronide of O-demethyl 17-hydroxy muraglitazar</td>
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<td>M18b</td>
<td>24</td>
<td>708</td>
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<td>M21</td>
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<td>317</td>
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<td>O-Demethyl O-dealkyl muraglitazar</td>
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</table>

*Identification of the oxidative metabolites of muraglitazar was described previously (Zhang et al., 2006).*

Bile. The metabolic profiles of pooled bile samples (3–4, 4–6, and 6–8 h after dose administration, pooled across all four subjects) are shown in Fig. 7A. The metabolic profiles of individual pooled bile samples (pooled from 3–8 h after dose administration) are shown in Fig. 7B. The metabolic profiles of bile samples were qualitatively similar between the interval pool sample and individual pool sample as well to the grand pool sample (data not shown). The major bile metabolites in the grand pool bile sample included the acyl glucuronide of muraglitazar (M13, 15% of the dose), glucuronides of O-demethyl 8-hydroxy muraglitazar (M17, 7% of the dose), 3 isomers, a, b, and c), glucuronides of hydroxy muraglitazar (M18, 6% of the dose, 3 isomers, a, b, and c), and the acyl glucuronide of O-demethyl muraglitazar (M20, 3% of the dose). The parent drug accounted for approximately 4% of the dose in the bile, suggesting an elimination mechanism of muraglitazar by direct biliary excretion. Oxidative metabolites (M10, M12, and M15) were minor components in the bile. These results indicated that glucuronidation was the major clearance pathway of muraglitazar in humans.

Feces. The metabolic profiles of feces were qualitatively similar between the two groups (Fig. 6B). The distribution of the radiactive metabolites in feces is listed in Table 3. The unchanged drug in feces accounted for approximately 23% of the dose. The major metabolites in feces of group 1 subjects were hydroxy muraglitazar (M10 and M11), O-demethyl muraglitazar (M15), O-demethyl hydroxy muraglitazar (M5), M8 (12,17-hydroxy muraglitazar), and oxazole ring-opening derivatives of muraglitazar (M9 and M16). The minor metabolites in feces included M1, M2, M3, M4, M6, M7, M8a, M12, M13, and M14. The fecal metabolite profile was similar to that found in the previous study (Zhang et al., 2006). The results from metabolite profiling of fecal homogenate would suggest that muraglitazar was extensively metabolized by oxidative pathways in humans; however, instead of oxidative metabolites, the glucuronides were the major drug-related components in the bile of humans.

Identification of Metabolites. The structures of metabolites were elucidated by LC/MS and LC/MS/MS analysis, and by the radiochromatographic and mass spectral comparisons to synthesized standards. Table 4 shows the characterization of proposed muraglitazar metabolites in humans. A total of 25 metabolites of muraglitazar were identified in human samples, including a number of glucuronic acid conjugates of muraglitazar and its oxidative metabolites (in bile, plasma, and urine).

Parent compound muraglitazar (P) showed a molecular ion [M + H]\(^{+}\) at m/z 517. Typical fragmentation patterns of muraglitazar in the full-scan MS/MS analysis showed cleavage at the benzylc C-N bond adjacent to the carbamate to give a fragment at m/z 292 and cleavage at the ether bond to give a fragment at m/z 186. These are also typical fragmentation patterns for muraglitazar metabolites. Among several prominent oxidative metabolites of muraglitazar in human feces, M10 and M11 were hydroxylated metabolites. Previous studies have described the identification of M10 and M11 by isolation from bioreactors and LC/MS/MS analyses (Zhang et al., 2006). Metabolites M10 and M11 were subsequently synthesized, characterized, and used as reference standards for comparison to metabolites formed in human plasma, urine, bile, and fecal samples in the current study. M10 showed a molecular ion [M + H]\(^{+}\) at m/z 533 (517 + 16) and major fragment ions at m/z 290 (308 – 18), 202 (186 + 16), and 184 (202 – 18) in LC/MS/MS analysis. M10 was assigned as 12-hydroxy muraglitazar based on LC/MS/MS analysis and HPLC retention time comparison to the synthetic standard. M11 showed a molecular ion [M + H]\(^{+}\) at m/z 533 and major fragment ions at m/z 308 and 202 by LC/MS/MS analysis. On the basis of LC/MS/MS analysis and com-
comparison to the synthetic standard, M11 was assigned as 17-hydroxy muraglitazar.

M2–M12, M14, M15, M16, and M21 had the same retention time and mass spectral fragmentation pattern as metabolites identified previously (Zhang et al., 2006). M13 was a major metabolite in human bile and urine, but minor in plasma. This metabolite had a molecular ion \([M + H]^+\) at \(m/z\) 693 and fragment ions at \(m/z\) 517 and 292 (Fig. 8A). M13 was assigned as the acyl glucuronide of muraglitazar. M17a showed a molecular ion \([M + H]^+\) at \(m/z\) 695 and fragment ions at \(m/z\) 677 (695 – 18), 519 (695 – 176), 501 (519 – 18), 484 (308 + 176), 378 (202 + 176), 308, and 202 (Fig. 8B), consistent with an ether glucuronide of \(O\)-demethyl hydroxy muraglitazar. The multiple isomers of M17 and M18 (labeled M17b,c and M18a,c) are probably due to the multiple hydroxylation sites on the muraglitazar molecule. M17 and M18 were assigned as glucuronide conjugates of \(O\)-demethyl hydroxy muraglitazar. M18a, M18b, and M18c showed the same molecular ion \([M + H]^+\) at \(m/z\) 709. M18a and M18c had fragment ions at \(m/z\) 691 (709 – 18), 533 (709 – 176), 515 (533 – 18), and 308 or 290 (308 – 18) (Fig. 9, A and C), consistent with the structure of an acyl glucuronide of hydroxy muraglitazar. M18b had fragment ions at \(m/z\) 533 (709 – 176), 484 (308 + 176), 378 (202 + 176), 308, and 202 (Fig. 9B), consistent with an ether glucuronide of hydroxy muraglitazar. The multiple isomers of M17 and M18 (labeled M17b,c and M18a,c) are probably due to the multiple hydroxylation sites on the muraglitazar molecule. M17 and M18 were assigned as glucuronide conjugates of \(O\)-demethyl hydroxy muraglitazar and hydroxy muraglitazar, respectively. M20 showed a molecular ion \([M + H]^+\) at \(m/z\) 679 and major fragment ions at \(m/z\) 661 (679 – 18), 519 (695 – 176), 501 (519 – 18), 308, 290, and 202 (Fig. 8, C and D), consistent with the structures of acyl glucuronides of \(O\)-demethyl hydroxy muraglitazar.

Fig. 8. MS/MS spectra of M13 (A), M17a (B), M17b (C), and M17c (D) from human bile. The samples were analyzed by a Finnigan LTQ ion trap LC/MS. LC/MS analysis was as described under Materials and Methods.
urine samples of subjects from both groups and showed a molecular ion \([\text{M} + \text{H}]^+\) at \(m/z\) 318, consistent with \(O\)-demethyl \(O\)-dealkyl muraglitazar. Figure 10 shows the proposed pathways for glucuronidation of muraglitazar in humans.

**Discussion**

Our previous human ADME study with a single 10-mg oral dose showed a low overall radioactivity recovery (64%) (Zhang et al., 2006). In addition, we have predicted that the glucuronidation would play an important role in elimination of muraglitazar in humans based on our observations in bile duct-cannulated rat studies. The purpose of this study was to examine the disposition, metabolism, and mass balance of \([14C]\)muraglitazar in healthy male human subjects after a single 20-mg oral dose with and without bile collection (groups 1 and 2). After oral administration of \([14C]\)muraglitazar, the recovery of radioactivity averaged 91% in feces for group 1 subjects without bile collection. In group 2 subjects, 40% of the radioactivity was recovered in the bile during the 3- to 8-h collection period and 51% of the radioactivity was recovered in feces. Only about 2 to 4% of radioactivity was excreted into urine for both groups of subjects. The results indicated that biliary excretion was the major elimination route for muraglitazar in humans.

Other procedures for bile collection included ultrasound-guided percutaneous fine needle puncture of the gallbladder (Hussaini et al., 1995) and surgical methods such as T-tube drainage (Burnstein et al., 1982; Cheng et al., 1994). All of these methods are complicated and involve surgery, and are, thus, not readily adaptable for use in drug metabolism studies. The present study used a simple and less invasive method for bile collection that was similar to the procedure described previously (Strasberg et al., 1990; Choudhuri et al., 1993). In this method, the terminal end of an oral-gastro-duodenal tube was fluoroscopically positioned at the vertical limb of the duodenal loop near the ampulla of Vater, and bile was collected via the tube upon application of mild suction. A previous study has demonstrated that duodenal bile was qualitatively similar to gallbladder bile in terms of biliary lipid contents, although duodenal bile was diluted by gastric and duodenal juice (Choudhuri et al., 1993). The bile collections were performed between 3 and 8 h after oral dose administration. The recovery in the 3- to 8-h bile accounted for approximately 40% of the radioactive dose. Although the bile collection with this method was limited to a relatively short period of time after dose administration, the method is simple and effective, and provided important additional information on the metabolic pathways and the excretion route of muraglitazar.

Different metabolic profiles of \([14C]\)muraglitazar were observed from subjects with and without bile collection. Bile contained the glucuronides of muraglitazar and its oxidative metabolites as the major drug-related components, but the feces from subjects with or without bile collection contained only oxidative metabolites and the parent compound. These data suggested that glucuronidation was a major pathway for the metabolic clearance of muraglitazar and that
this pathway was missed in the previous human ADME study of \([^{14}C]\)muraglitazar with traditional urine and feces collection (Zhang et al., 2006). Our preliminary data indicated that multiple cytochrome \(P450\) and \(UDP\)-glucuronosyltransferase enzymes were involved in the oxidation and glucuronidation of muraglitazar, and these results will be communicated separately. For many drugs without any functional group for direct conjugation, glucuronidation normally follows an oxidation step such as hydroxylation and \(O\)-demethylation. Recent examples have also shown that glucuronide metabolites estradiol 17\(\beta\)-glucuronide, diclofenac glucuronide, and gemfibrozil glucuronide could be further metabolized by cytochrome \(P450\)-mediated oxidation (Kumar et al., 2002; Delaforge et al., 2005; Ogilvie et al., 2006). It is, thus, possible that the glucuronide conjugates of the oxidative metabolites of muraglitazar found in the bile were actually formed from muraglitazar glucuronide.

The \(C_{\text{max}}\) for both total radioactivity and unchanged muraglitazar was reached at 1.5 h postdose, indicating rapid absorption of muraglitazar. At \(T_{\text{max}}\), unchanged muraglitazar accounted for 97% of the plasma total radioactivity, and the AUC of unchanged muraglitazar was 77% of the AUC of the plasma total radioactivity, indicating that metabolites were relatively minor circulating components. The metabolic profiles of 1-h plasma samples showed that muraglitazar was the major drug-related component (92% for group 1 and 89% for group 2). By 24 h postdose, although the concentration of plasma radioactivity had declined significantly, muraglitazar was still the predominant radioactive peak for both groups. The metabolites observed in plasma included an acyl glucuronide of muraglitazar (M13), glucuronides of hydroxy muraglitazar (M18, three isomers), a glucuronide of \(O\)-demethyl muraglitazar (M20), and an oxazole ring-opening derivative of muraglitazar (M16), but none of these metabolites represented more than 5% of the total radioactivity in 1-h plasma samples. These glucuronide metabolites would almost certainly be pharmacologically inactive metabolites based on structure and activity correlation analysis, and a preliminary result indicated that M16 was an inactive metabolite (D. Zhang, personal communication). Therefore, the pharmacological activities of muraglitazar would be dictated by the parent compound and not by its minor circulating metabolites. There was a difference in AUC values between these two groups. However, the approximately 19% higher AUC value may be due to the higher \(C_{\text{max}}\) values in group 1 subjects (the origin of the differences in \(C_{\text{max}}\) values between groups is unclear). Because \(C_{\text{max}}\) occurs before the bile collection period, this observation tends to negate the idea that AUC differences found in the groups were a result of bile collection. Unfortunately, there were not enough plasma samples taken in the time period directly after the bile collection to fully describe the plasma concentration versus time profile in that region and allow for delineation of the full effects of bile collection on plasma drug concentration.

Fig. 10. Proposed pathways for glucuronidation of muraglitazar in humans.
Biliary elimination and intestinal hydrolysis of glucuronide metabolites of muraglitazar would present the potential for enterohepatic circulation. Several lines of evidence support such a hypothesis. The parent compound and the acyl glucuronide of the parent represent approximately 50% of the bile excretion. The intestinal hydrolysis of the acyl glucuronide would lead to approximately half the drug-related material in fecal homogenates being the parent compound, given an assumption that the 3- to 8-h bile collection represented the overall biliary excretion. This might even underestimate the amount of the parent in feces, because there could be a small fraction of unab sorbed dose. However, the parent compound only represents 20 to 25% of fecal excretion of radioactivity from the subjects without bile collection. These data suggest that muraglitazar and its glucuronide were further metabolized to oxidative metabolites. Indeed, the total oxidative metabolites (oxidative metabolites plus their glucuronide conjugates) in the 3- to 8-h bile sample of group 2 accounted for only 42% of sample radioactivity, whereas the total oxidative metabolites in the fecal samples of group 1 accounted for about 75% of sample radioactivity. The corresponding increases of individual oxidative metabolites in the fecal samples of group 1 accounted for about 75% of sample radioactivity. The corresponding increases of individual oxidative metabolites in the subjects without bile collection also supported the above analysis. These differences could best be explained by an enterohepatic circulation process of biliary excretion of the glucuronides of muraglitazar and its metabolites, hydrolysis of the glucuronides by bacterial β-glucuronidase, and reabsorption of the aglycones, followed by further metabolism before the oxidative metabolites were finally excreted into feces. The above discussion suggested a low level of enterohepatic circulation of muraglitazar compared with other carboxylic acid-containing drugs (Parkar et al., 1980; Pollack and Brouwer, 1991; Tabata et al., 1995).

The stability of acyl glucuronides depends on many factors, such as pH, temperature, and the nature of the aglycone. These metabolites may not be stable at physiological pH, and storage can result in free acglycone by hydrolysis and/or rearrangement to positional isomers by acyl migration (Faed, 1984; Spahn-Langguth and Benet, 1992). Our studies showed that the acyl glucuronide of muraglitazar was stable at room temperature in samples acidified immediately after collection and stored at −20°C.

In summary, the present study demonstrated different pathways of metabolic clearance of [14C]muraglitazar from human subjects with and without bile collection. Glucuronidation was demonstrated to be a major clearance pathway of muraglitazar in subjects with bile collection, and this major pathway was missed in the previous human ADME study with only conventional collection of urine and feces.

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References


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