Metabolism and Disposition of $^{14}$C-labeled Peliglitazar in Humans

Lifei Wang, Carey Munsick, Sean Chen, Samuel Bonacorsi, Peter T Cheng, W. Griffith Humphreys, and Donglu Zhang

Pharmaceutical Candidate Optimization (LW, WGH, and DZ), Discovery medicines and Clinical Pharmacology (CM), Discovery Chemistry (SC, PTC, and SB) Discovery & Development, Bristol-Myers Squibb, Princeton, NJ 08543
Running title: Metabolism of $^{14}$C-labeled peliglitazar in humans

Corresponding Author: Lifei Wang, PO Box 4000, Pharmaceutical Candidate Optimization, Discovery & Development, Bristol-Myers Squibb, Princeton, New Jersey 08543-4000. Phone: 609-252-5657; Fax: 609-252-6802

E-mail: Lifei.Wang@bms.com

Texte pages: 21

Tables: 5

Figures: 6

References: 20

Data supplement figures: 7

Number of words in abstract: 227

Number of words in introduction: 237

Number of words in discussion: 816

Abbreviations: AUC, area under the plasma concentration vs time curve; HPLC, high pressure liquid chromatography; LC/MS, liquid chromatography/mass spectrometry; PPAR, peroxisome proliferator-activated receptors; PEG-400, polyethylene glycol 400; TFA, trifluoroacetic acid.
ABSTRACT

The metabolism and disposition of dual $^{14}$C-labeled peliglitazar, a dual alpha/gamma PPAR 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 hour and the elimination half-life ($t_{1/2}$) was about 3.5 hours. The exposure to drug metabolites ($C_{max}$ and AUC) was not significantly different between the two groups. The parent compound and its 1-$O$-$\beta$-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 radioactive dose was recovered in feces (94% for Group 1 and 32% for Group 2). Approximately 24% of 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) 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 (Taskinen, 2003; Yajima et al., 2004; Torra et al., 2001). 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; Packard, 1998; Despres, 2001) and gemfibrozil (Spencer and Barradell, 1996), whereas PPARγ is the target of the thiazolidinedione (Mudaliar and Herry, 2001) class of antidiabetic drugs such as rosiglitazone (Balfour and Plosker, 1999; Cheng-Lai and Levine, 2000; Goldstein, 2000) and pioglitazone (Gillies and Dunn, 2000).

Pelgilitazar, (S)-2-(((4-methoxyphenoxy)carbonyl)(1-(4-(2-(5-methyl-2-phenyloxazol-4-yl)ethoxy)phenyl)ethyl)amino)acetic acid (Figure 1), is a novel dual alpha/gamma PPAR activator. It has been shown that pelgilitazar has both glucose and lipid lowering effects when tested in animal models of diabetes and dyslipidemia and in patients with diabetes. In order to further explore the metabolism, disposition, and pharmacokinetics of pelgilitazar, especially the role
of glucuronidation and biliary elimination, a single 10 mg oral dose of $[^{14}C]$peligitazar was administered to subjects with or without bile collection.
MATERIALS AND METHODS

Radiolabeled drug and chemicals

Two versions of [14C]-labeled peliglitazar (Figure 1) were synthesized separately at Bristol-Myers Squibb and mixed at a 14C ratio of 1:1 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 similar procedures as described previously (Wang et al., 2006; Zhang et al., 2006). 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 principles 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 prior to participation in the study. All subjects were in good health as determined by medical history, physical examination, and clinical laboratory tests conducted prior to study based on inclusion and exclusion criteria.
Ten healthy male subjects, aged 18 to 45 years, were assigned to two groups, Groups 1 (6 subjects) and Group 2 (4 subjects). After at least an 8 h overnight fast, each subject (Groups 1 and 2) received a single dose of 10 mg [\(^{14}\text{C}\)]peliglitazar containing 102.6 μCi 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 prior to 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 \(\leq 1\%\) of administered radioactivity.

**Collection of blood, bile, urine, and feces**

Blood samples for pharmacokinetic analysis were drawn (5 mL each) prior to 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 K\(_3\)EDTA as the anticoagulant. Plasma was prepared by centrifugation at approximately 1000\(g\) for 15 min at 4\(^{\circ}\)C. Acetic acid was added to plasma to a final concentration of 5\% (v/v) (0.83 M). Immediately following processing, all plasma samples were frozen and stored at -20\(^{\circ}\)C.

Bile samples were collected from Group 2 subjects using suction during 3-4, 4-6, and 6-8 h periods post dose. 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 hour post dose. 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 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. Acetic acid was added after each collection to a final concentration of 5% (v/v) (0.83 M). 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 prior to processing; a volume of 5% (v/v) acetic acid (0.83 M) in 50% reagent ethanol was added to each sample to form an approximate 20% (w/w) feces/solvent homogenate; and 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 (LSC) after an aliquot of each sample was combusted. Sample combustion was performed by using a Model A0387 sample
oxidizer (PerkinElmer Life Sciences, Inc., Boston, Massachusetts). The resulting $^{14}$CO$_2$ was trapped with Carbo-Sorb E (PerkinElmer Life Sciences, Inc.), mixed with Permafluor E+ (PerkinElmer Life Sciences, Inc.) scintillation fluid, and the radioactivity was quantified using liquid scintillation counting. The combustion efficiency was determined prior to 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 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 spiked fecal homogenate to determine the combustion efficiency value. Combustion efficiency was within 100% ± 5% (100% ± 10% for feces). Samples were analyzed for radioactivity by counting for 5 min in a Model LS 6500 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, California). 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 percent 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, $C_{\text{max}}$, and the time to reach peak concentration, $T_{\text{max}}$, were recorded directly from experimental observations. The area under the plasma concentration vs 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 equivalents of peliglitazar, in the terminal phase of each plasma concentration vs 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 $\text{AUC}_{(0-12 \text{ h})}$ to estimate $\text{AUC}_{(0-\infty)}$.

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 a mixture of 1 mL methanol and 3 mL acetonitrile and mixed on a vortex mixer. The mixtures were centrifuged at 2000g at 10°C 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 based on the radioactivity determination of before and after extraction. The combined supernatants were concentrated under a steam of nitrogen, the residues were then 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, centrifuged at 2000g 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 h, 4-6 h, 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 the 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% of mobile phase B, vortexed, and centrifuged at 2000g for 10 min, and 200 μL of the supernatant was used for HPLC analysis.
Two grand pools of fecal homogenate samples (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 methanol and 3 ml 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 steam 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, 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 ultraviolet (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 μ 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 sciences, Meriden, CT) 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 Speed-Vac (Savant) and counted for 10 min per well with a TopCount analyzer (PerkinElmer Sciences, Meriden, CT) to quantify radioactivity. Biotransformation profiles were prepared by plotting the resulting net CPM values vs 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 (ThermoFinnigan, San Jose, CA). The samples were analyzed by positive mode electrospray ionization (ESI). 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-3 120Å column. The mobile phase flow rate was 0.28 mL/min. The gradient used was as 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 0-5 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 peliglitazar. 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]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 about 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 6-8 h interval samples (about 22% of the radioactive dose) probably due to gallbladder emptying stimulated by administration of cholecystokinin at 7 h post dose. Approximately 1.5% of the radioactive dose was found in the urine for both groups.

Pharmacokinetic parameters of total radioactivity

Following oral administration, the Cmax of total radioactivity was determined at 1.1 h post dose in both groups. The mean half-life of radioactivity was about 3.5 h for both groups (Table 2). The mean AUC0-12 value from subjects with bile collection was similar as 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 [\textsuperscript{14}C]peliglitazar

\textbf{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 65-100\% of the plasma radioactivity. In the 1 h sample visible metabolites included glucuronide conjugated metabolites M14, M15, M15a, M17, and oxidative metabolite M3. Except 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.

\textbf{Bile.} The metabolic profiles of pooled bile samples (grand pool, 3-4, 4-6, and 6-8 h) are shown in Figure 3. The metabolic profiles of bile samples were qualitatively similar between the interval-pooled sample and individual pooled sample (profiles not shown) as well to the grand pool sample. The major bile metabolites in the grand pooled bile sample included the acyl glucuronide of peliglitazar (20\% of the dose, 3 isomers M14, M14a, and M14b), glucuronides of hydroxy peliglitazar (1.5\% of the dose, 3 isomers M15, M15a, and M15b), 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 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 (Figure 4). The relative distribution of the radioactive metabolites in feces is listed in Table 4. Unchanged drug in feces accounted for about 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 to the synthesized standards. Table 5 shows the structures of proposed peliglitazar metabolites.

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 to a synthetic standard.

M2 showed a molecular ion \([\text{M+H}]^+\) at m/z 218 and major fragment ions at m/z 172. M2 was assigned as 5-methyl-2-phenoxazyl-4-acetic acid based on LC/MS/MS analysis and HPLC retention time comparison to a synthetic standard.

M3 was a minor metabolite in human samples and its molecular ion \([\text{M+H}]^+\) and fragment ions were not obtained under the study conditions. M3 was assigned as O-dealkyl peliglitazar based on HPLC retention time comparison to a synthetic standard.

M4, M5, and M6 had the same mass spectral fragmentation pattern, showed a molecular ion \([\text{M+H}]^+\) 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 to the synthetic standards, M4, M5, and M6 were assigned as hydroxy metabolites of peliglitazar.

M7 had a molecular ion \([\text{M+H}]^+\) 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 to the synthetic standard, M7 was assigned as 12-hydroxy O-demethyl peliglitazar.
M8 had a molecular ion \([\text{M+H}]^+\) 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 proposed as 9-hydroxy O-demethyl peliglitazar.

M10 yielded an ammonium adduct molecular ion \([\text{M+NH}_4]^+\) 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 peligalitazar (MW = 520).

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

M15 showed the same molecular ion \([\text{M+H}]^+\) 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), 322 (306+16) and 304 (322-18). M15a was also assigned as an ether glucuronide of hydroxy peliglitazar.

M15b showed a 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), 547 (723-176), 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+176), consistent with a glucuronide of demethyl parent drug. The major fragment ions were m/z 675 (693-18), 517 (693-176), and 306. M17 was assigned as an acyl glucuronide of O-demethyl peliglitazar, although the possibility as an ether glucuronide can not be ruled out.

Summary, sixteen 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 Figure 6.
DISCUSSION

The purpose of this study was to investigate the mass balance, disposition, and metabolism of dual labeled \[^{14}\text{C}]\text{pelaglitazar}\) in healthy male human subjects following a single 10 mg oral dose with and without bile collection (Group 1 and 2). Since an \(O\)-dealkylation reaction was observed in studies with a structurally related analog muraglitazar that split the molecule into two large fragments and a the \(O\)-dealkylation reaction was observed in incubations of pelaglitazar in human hepatocyte (data not shown), dual labeled \[^{14}\text{C}]\text{pelaglitazar}\) was used to ensure the complete disposition profile of pelaglitazar 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 due to a partial loss of the radioactivity in the bile collection tubing. The exact reason for the low recovery remains unclear.

In Group 2 subjects, about 24\% of the radioactivity was recovered in the bile during the 3-8 h collection period and 32\% of the radioactivity was recovered from feces. In both groups, only about 1.5\% of radioactivity was excreted into urine. The results indicated that biliary excretion was the major elimination route for pelaglitazar and its metabolites in humans.

The \(C_{\text{max}}\) of total radioactivity was reached at 1.1 h post dose, indicating rapid absorption of pelaglitazar. The metabolic profiles of 1 h plasma sample showed
that peliglitazar (38-67%) and 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 post dose, 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 time after dose administration, the recovery in the 3-8 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 3-8 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 about 23% of the radioactive dose. These results indicated that: 1) glucuronide conjugation was an important metabolic pathway for peliglitazar; 2) the glucuronide conjugates were excreted into bile as the major elimination route for peliglitazar in humans. While conjugation seems to be the predominant clearance pathway, but hydrolysis sets up a situation for enterohepatic recirculation of peliglitazar and metabolites. While reabsorption of these compounds undoubtedly happens to some extent, the low levels of oxidative metabolites in circulation argue against significant recirculation of peliglitazar metabolites. Peliglitazar in feces could be from non-absorbed 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 similar molecular structure to peliglitazar, was extensively metabolized in humans and the major metabolite was also an acyl glucuronide eliminated in the bile (Wang, et. al, 2007; Zhang, et al, 2007a; Zhang, et al, 2007b). However, the major circulating component in humans was parent compound after oral administration of muraglitazar with very little glucuronide conjugates detected (Wang, et al, 2007). The stability of peliglitazar acyl glucuronide was a concern as 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°C. Other experiments to look at the stability of peliglitazar glucuronide were also performed and the results reported separately (Zhang et al., 2010).

In summary, the present study demonstrates that [14C]peliglitazar was rapidly absorbed after oral administration. Most of the radioactive dose was recovered in feces or bile. [14C]Peliglitazar was extensively metabolized in humans. Parent and a glucuronide conjugate were 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.
ACKNOWLEDGEMENTS

We would like to thank Dr. Vijayalakshmi Surya Pratha from Clinical Applications Laboratories, Inc. for contributing to the clinical study.
REFERENCES


Figure legends:

Figure 1 Structure of peliglitazar.

Figure 2 Metabolite profiles of the pooled plasma samples of Group 1 following single oral administration of 10 mg [14C]peliglitazar to humans at 1, 4, 12, and 24 h. 1, 2, 3, ...., and P represents M1, M2, M3, ...., and parent compound.

Figure 3 Metabolite profiles of the pooled bile samples of Group 2 following single oral administration of 10 mg [14C]peliglitazar to humans. 1, 2, 3, ...., and P represents M1, M2, M3, ...., and parent compound.

Figure 4 Metabolite profiles of pooled fecal samples of Group1 and 2 following single oral administration of 10 mg [14C]peliglitazar to humans. 1, 2, 3, ...., and P represents M1, M2, M3, ...., and parent compound.

Figure 5 MS/MS spectra of M14a (A), M14 (B), and M14b (C) from human bile. The samples were analyzed by LTQ ion trap LC/MS. LC/MS analysis was done described under Material and Methods.

Figure 6 Proposed metabolic pathways of peliglitazar in humans.
Table 1: Recovery of radioactivity in urine, feces, and bile following administration of single 10 mg oral dose of [14C]peliglitazar

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<th>Subject No</th>
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<td>Urine</td>
<td>Feces</td>
<td>Total</td>
<td>Urine</td>
<td>Feces</td>
<td>Bile</td>
<td>Total</td>
</tr>
<tr>
<td>0002</td>
<td>1.67</td>
<td>101.1</td>
<td>102.8</td>
<td>0001</td>
<td>2.10</td>
<td>24.2</td>
<td>27.1</td>
</tr>
<tr>
<td>0004</td>
<td>1.08</td>
<td>91.7</td>
<td>92.8</td>
<td>0003</td>
<td>1.27</td>
<td>34.9</td>
<td>42.2</td>
</tr>
<tr>
<td>0006</td>
<td>0.73</td>
<td>98.8</td>
<td>99.5</td>
<td>0005</td>
<td>0.98</td>
<td>22.8</td>
<td>14.5</td>
</tr>
<tr>
<td>0007</td>
<td>0.99</td>
<td>88.9</td>
<td>89.9</td>
<td>0008</td>
<td>1.03</td>
<td>45.4</td>
<td>13.9</td>
</tr>
<tr>
<td>0009</td>
<td>2.81</td>
<td>88.3</td>
<td>91.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0010</td>
<td>1.55</td>
<td>93.3</td>
<td>94.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean</td>
<td>1.47</td>
<td>93.7</td>
<td>95.2</td>
<td>Mean</td>
<td>1.35</td>
<td>31.9</td>
<td>24.4</td>
</tr>
<tr>
<td>SD</td>
<td>0.74</td>
<td>5.2</td>
<td>5.0</td>
<td>SD</td>
<td>0.52</td>
<td>10.5</td>
<td>13.3</td>
</tr>
</tbody>
</table>

SD: standard deviation
Table 2: Summary of pharmacokinetic parameters of total radioactivity in human plasma after a single oral dose of 10 mg [¹⁴C]peliglitazar (data presented as mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>$C_{\text{max}}$ (ng/mL)</th>
<th>$\text{AUC}_{(0-12\text{ h})}$ (ng·h/mL)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>$t_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Subjects</td>
<td>251 ± 47</td>
<td>958 ± 339</td>
<td>1.1 ± 0.4</td>
<td>3.5 ± 1.1</td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>257 ± 56</td>
<td>1035 ± 328</td>
<td>1.1 ± 0.5</td>
<td>3.6 ± 1.1</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>242 ± 35</td>
<td>844 ± 371</td>
<td>1.0 ± 0.4</td>
<td>3.3 ± 1.2</td>
</tr>
<tr>
<td>(n = 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All p > 0.05, t-test, Group 1 vs Group 2
Table 3: Plasma radioactivity concentration and relative distribution of radioactive metabolites in pooled human plasma samples at 1, 4, 12, and 24h following single oral administration of 10 mg [14C]peliglitazar

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Plasma radioactivity concentration (ng equivalents of parent drug/g)</th>
<th>Metabolite distribution in plasma (% of sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>4 h</td>
</tr>
<tr>
<td>Group 1</td>
<td>216</td>
<td>88</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>3.8</td>
<td>3.5</td>
</tr>
<tr>
<td>M14</td>
<td>47.3</td>
<td>26.0</td>
</tr>
<tr>
<td>M15a</td>
<td>2.8</td>
<td>1.3</td>
</tr>
<tr>
<td>M15</td>
<td>2.5</td>
<td>1.1</td>
</tr>
<tr>
<td>M17</td>
<td>2.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Parent</td>
<td>38.2</td>
<td>67.2</td>
</tr>
<tr>
<td>Total</td>
<td>97.4</td>
<td>100</td>
</tr>
</tbody>
</table>

a: M4, M5, M6, M14a, M14b, and M15b were detected by LC/MS in trace amounts in samples of 1 h or 4 h. The molecular ion and fragments of M3 were not obtained under these study conditions. M3 was assigned as O-dealkyl peliglitazar by comparing the HPLC retention time with the synthetic metabolite standard.

b: NA = not analyzed by LC/MS; negligible amounts of radioactivity.

c: Trace = trace amounts by radioactivity and detectable by LC/MS.
Table 4: Relative distribution of radioactive metabolites in pooled human bile (3-8 h), urine (0-240 h), and feces (0-240 h) following single oral administration of 10 mg [14C]peliglitazar

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Group 1 (% of dose)</th>
<th>Group 2 (% of dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feces</td>
<td>Urine</td>
</tr>
<tr>
<td>M1</td>
<td>14.6</td>
<td>trace</td>
</tr>
<tr>
<td>M2</td>
<td>0.88</td>
<td>0.16</td>
</tr>
<tr>
<td>M3</td>
<td>4.38</td>
<td>0.16</td>
</tr>
<tr>
<td>M4</td>
<td>6.66</td>
<td>trace</td>
</tr>
<tr>
<td>M5</td>
<td>0.88</td>
<td>trace</td>
</tr>
<tr>
<td>M6</td>
<td>1.58</td>
<td>trace</td>
</tr>
<tr>
<td>M7</td>
<td>6.13</td>
<td>trace</td>
</tr>
<tr>
<td>M8</td>
<td>1.23</td>
<td>trace</td>
</tr>
<tr>
<td>M10</td>
<td>5.52</td>
<td></td>
</tr>
<tr>
<td>M14</td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>M14a</td>
<td></td>
<td>0.29</td>
</tr>
<tr>
<td>M14b</td>
<td></td>
<td>3.34</td>
</tr>
<tr>
<td>M15</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>M15a</td>
<td></td>
<td>0.48</td>
</tr>
<tr>
<td>M15b</td>
<td></td>
<td>trace</td>
</tr>
<tr>
<td>M17</td>
<td></td>
<td>1.8</td>
</tr>
<tr>
<td>Parent</td>
<td>47.2</td>
<td>trace</td>
</tr>
<tr>
<td>Other</td>
<td>4.58</td>
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</tr>
<tr>
<td>Total</td>
<td>93.7</td>
<td>1.47</td>
</tr>
</tbody>
</table>

* Metabolites M9, M11, M12, M13, and M16 were observed in animal samples but not in human samples.

* Metabolite was detected by LC/MS but in trace amounts by radioactivity.
Table 5: Proposed structures of [14C]peliglitazar metabolites in human samples.

<table>
<thead>
<tr>
<th>Peak</th>
<th>RT (min)</th>
<th>Proposed structure</th>
<th>Descriptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>65.5</td>
<td><img src="image" alt="Structure P" /></td>
<td>Peliglitazar</td>
</tr>
<tr>
<td>M1</td>
<td>48.4</td>
<td><img src="image" alt="Structure M1" /></td>
<td>O-Demethyl peliglitazar</td>
</tr>
<tr>
<td>M2</td>
<td>18.5</td>
<td><img src="image" alt="Structure M2" /></td>
<td>O-dealkyl peliglitazar</td>
</tr>
<tr>
<td>M3</td>
<td>21</td>
<td><img src="image" alt="Structure M3" /></td>
<td>O-Dealkyl peliglitazar</td>
</tr>
<tr>
<td>M4</td>
<td>39.5</td>
<td><img src="image" alt="Structure M4" /></td>
<td>12-Hydroxy peliglitazar</td>
</tr>
<tr>
<td>M5</td>
<td>42.3</td>
<td><img src="image" alt="Structure M5" /></td>
<td>17-Hydroxy peliglitazar</td>
</tr>
<tr>
<td>M6</td>
<td>46.8</td>
<td><img src="image" alt="Structure M6" /></td>
<td>9-Hydroxy peliglitazar</td>
</tr>
<tr>
<td>M7</td>
<td>27.6</td>
<td><img src="image" alt="Structure M7" /></td>
<td>12-Hydroxy O-demethyl peliglitazar</td>
</tr>
</tbody>
</table>
Table 5- Continued: Proposed structures of $[^{14}C]$peliglitazar metabolites in human samples

<table>
<thead>
<tr>
<th>Peak</th>
<th>RT (min)</th>
<th>Proposed structure</th>
<th>Descriptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>M8</td>
<td>32.1</td>
<td><img src="image1" alt="Proposed structure" /></td>
<td>9-Hydroxy $O$-demethyl peliglitazar</td>
</tr>
<tr>
<td></td>
<td></td>
<td><img src="image2" alt="Proposed structure" /></td>
<td>Ring-opened metabolite of peliglitazar</td>
</tr>
<tr>
<td>M14a, M14, M14b</td>
<td>41.6-45</td>
<td><img src="image3" alt="Proposed structure" /></td>
<td>Acyl glucuronide of peliglitazar</td>
</tr>
<tr>
<td>M15a, M15,</td>
<td>24.7-26</td>
<td><img src="image4" alt="Proposed structure" /></td>
<td>Ether glucuronide of hydroxy peliglitazar</td>
</tr>
<tr>
<td>M15b</td>
<td>27.3</td>
<td><img src="image5" alt="Proposed structure" /></td>
<td>Acyl glucuronide of hydroxy peliglitazar</td>
</tr>
<tr>
<td>M17</td>
<td>32.2</td>
<td><img src="image6" alt="Proposed structure" /></td>
<td>Glucuronide of $O$-demethyl peliglitazar</td>
</tr>
</tbody>
</table>
Figure 1.

Peliglitazar
* indicates C-14 labels
Figure 2.
Figure 3.

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Figure 4.
Figure 5.
Figure 6.
Data Supplements:

Figure S1. The ion chromatogram, MS, and MS2 spectra of authentic standard of peliglitazar metabolite M1.

Figure S2. The ion chromatogram, MS, and MS2 spectra of authentic standard peliglitazar metabolite M2.

Figure S3. The ion chromatogram, MS, and MS2 spectra of authentic standard peliglitazar metabolite M3.

Figure S4. The ion chromatogram, MS, and MS2 spectra of authentic standard peliglitazar metabolite M4.

Figure S5. The ion chromatogram, MS, and MS2 spectra of authentic standard peliglitazar metabolite M5.

Figure S6. The ion chromatogram, MS, and MS2 spectra of authentic standard peliglitazar metabolite M7.

Figure S7. The MS2 spectra of peliglitazar metabolites M15, M15a, M15b, and M17.
Figure S1

NL: 2.02E6
m/z= 516.50-517.50 F:
ITMS + c ESI Full ms [140.00-1000.00] MS
BMS-712079-10ul-092904
Figure S2

NL: 7.23E7
m/z= 217.50-218.50 F:
ITMS + c ESI Full ms [140.00-1000.00] MS
BMS-374454-5ul-092904

Figure S2
Figure S3
Figure S5
Figure S6

NL: 3.79E6
m/z= 532.50-533.50 F:
ITMS + c ESI Full ms [140.00-1000.00] MS
BMS-719443-5ul-092904
Figure S7

A: MS/MS of m/z 723 (M15a)

B: MS/MS of m/z 723 (M15)

C: MS/MS of m/z 723 (M15b)

D: MS/MS of m/z 693 (M17)