IN VITRO AND IN VIVO DRUG-DRUG INTERACTION STUDIES TO ASSESS THE EFFECT OF ABIRATERONE ACETATE, ABIRATERONE, AND METABOLITES OF ABIRATERONE ON CYP2C8 ACTIVITY

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Nonstandard abbreviations

APR       Apparent partition ratio
GMR  Geometric mean ratio
OATP  Organic anion transporting polypeptide
Abstract

Abiraterone acetate, the prodrug of the cytochrome P450 (CYP) C17 inhibitor abiraterone, plus prednisone is approved for treatment of metastatic castration-resistant prostate cancer. We explored whether abiraterone interacts with drugs metabolized by CYP2C8, an enzyme responsible for the metabolism of many drugs. Abiraterone acetate and abiraterone and its major metabolites, abiraterone sulfate and abiraterone sulfate N-oxide, inhibited CYP2C8 in human liver microsomes, with IC₅₀ values that were near or below the peak total concentrations observed in patients with mCRPC (IC₅₀ values: 1.3–3.0 µM, 1.6–2.9 µM, 0.044–0.15 µM, and 5.4–5.9 µM, respectively). CYP2C8 inhibition was reversible and time-independent. To explore the clinical relevance of the in vitro data, an open-label, single-center study was conducted in 16 healthy male subjects who received a single 15-mg dose of the CYP2C8 substrate pioglitazone on day 1 and again at 1 h after abiraterone acetate 1000 mg on day 8. Plasma concentrations of pioglitazone, its active M-III (keto derivative) and M-IV (hydroxyl derivative) metabolites, and abiraterone were determined for up to 72 h after each dose. Abiraterone acetate increased exposure to pioglitazone; the geometric mean ratio (day 8/day 1) was 125 (90% CI, 99.9–156) for Cₘₐₓ and 146 (90% CI, 126–171) for AUCₗₐₛₜ. Exposure to M-III and M-IV was reduced by 10 to 13%. Plasma abiraterone concentrations were consistent with previous studies. These results show that abiraterone only weakly inhibits CYP2C8 in vivo.
Introduction

Abiraterone acetate in combination with prednisone is approved for the treatment of patients with metastatic castration-resistant prostate cancer (mCRPC) in the pre- and postchemotherapy settings based on two pivotal phase 3 trials that demonstrated a significant survival benefit versus prednisone alone (de Bono et al., 2011; Fizazi et al., 2012; Rathkopf and Scher, 2013; Ryan et al., 2013; Ryan et al., 2015). Abiraterone acetate is the prodrug of abiraterone, which selectively blocks the cytochrome P450 C17 (CYP17) complex that is required for androgen biosynthesis (Barrie et al., 1994; Potter et al., 1995). CYP17 is expressed in testicular, adrenal, and prostatic tumor tissues, and therefore abiraterone inhibits gonadal, extragonadal, and intratumoral androgen biosynthesis, resulting in profound reductions in circulating testosterone levels beyond those achieved by surgical or medical castration (Massard and Fizazi, 2011; Ryan and Tindall, 2011).

Cytochrome P450 enzymes play a central role in the metabolism of a wide range of drugs and chemical carcinogens (Guengerich 1988). CYP450 2C8 (CYP2C8), in particular, has emerged relatively recently as an important enzyme that is responsible for metabolism of a diverse group of drugs in humans, including repaglinide, pioglitazone, rosiglitazone, paclitaxel, dasabuvir, and the androgen signaling inhibitor enzalutamide (Daily and Aquilante, 2009; Gibbons et al., 2015). Available data suggest that genetic variation in CYP2C8 can alter drug disposition in vivo (Kirchheiner et al., 2006; Niemi et al., 2003; Niemi et al., 2005). Given the role of CYP2C8 in drug metabolism, we designed a series of in vitro studies to evaluate the potential of abiraterone acetate and abiraterone and its major human metabolites (Supplemental Figure 1) to inhibit CYP2C8. Repaglinide is a preferred substrate for CYP2C8 and is transported via organic anion
transporting polypeptide (OATP1B1) in vivo (Kalliokoski et al., 2008); because this raises the possibility that OATP1B1 may influence drug metabolism by CYP2C8 in the in vitro and in vivo setting, we also assessed the effects of abiraterone and its metabolites on hepatic uptake of OATP1B1 substrates.

Based on the results of the in vitro investigations on CYP2C8 and OATP1B1, we conducted a clinical study to further evaluate the effect of abiraterone on CYP2C8 activity after a single dose of abiraterone acetate in healthy volunteers. Pioglitazone was selected as the CYP2C8 substrate probe because it undergoes extensive hepatic metabolism by hydroxylation of aliphatic methylene groups predominantly mediated via CYP2C8 (Jaakkola et al., 2006b), and its disposition is not affected by the polymorphism of the SLCO1B1 gene that encodes OATP1B1 (Kalliokoski et al., 2008). Two active pioglitazone metabolites, M-III and M-IV (Supplemental Figure 2), which contribute to the long duration of glucose-lowering efficacy of pioglitazone (Eckland and Danhof, 2000), were also measured. Multiple cytochrome P-450 isoenzymes are involved in the metabolism of pioglitazone to M-IV, but the most important of these are CYP2C8/9 and, to a lesser extent, CYP3A4. M-IV can be further oxidized to form M-III (Eckland and Danhof 2000). Herein, we report the effects of abiraterone on CYP2C8 in in vitro and clinical studies.
Materials and Methods

Reagents and supplies. For the in vitro CYP2C8 assays, abiraterone acetate (Batch # 35430017), abiraterone (Batch # 342293), and quercetin (Batch # 30466093) were used from internal stocks. For the OATP1B1 assays, abiraterone (PbOEL3a) was used from internal stocks. For both the CYP2C8 and OATP1B1 assays, abiraterone sulfate (Batch # 35616973) and abiraterone sulfate N-oxide (Batch # 39333595) were used from internal stocks. Rifampicin (Batch # EXTE_0201_210_1) and cyclosporin A (Batch # 39195100) were used from internal stocks. Amodiaquine, DMSO, 3H-estradiol 17β-D-glucuronide (E2-17G), and 3H-repaglinide were purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. Gemfibrozil 1-O-β-glucuronide was purchased from Toronto Research Chemicals, Toronto, Ontario, Canada. The NADPH-regenerating system (NRS) was obtained from BD Biosciences, Woburn, MA, USA. Cryopreserved human hepatocytes were obtained from CellzDirect – Invitrogen (Thermo Fisher Scientific Inc., Grand Island, NY, USA).

The BD Gentest™ was purchased from BD Biosciences. The Strata™ Impact protein precipitation 96-well plates were purchased from Phenomenex® Corp., Torrance, CA, USA. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was carried out on a Shimadzu binary 10AD-vp liquid chromatography pump system (Shimadzu Scientific Instruments, Columbia, MD, USA) with Leap CTC PAL autosampler (Leap Technologies, Carrboro, NC, USA) coupled to a Sciex API 4000™ triple quadrupole mass spectrometer (Sciex, Foster City, CA, USA). The swinging bucket centrifuge (Eppendorf 5417R) was obtained from Eppendorf Biotools, Ontario, Canada. The Xbridge C18 column was obtained from Waters Corporation (Milford, MA, USA), and the API4000, with a Turbo-Ionspray™ interface was
obtained from was obtained from Sciex (Ontario, Canada). The Luna Phenylhexyl column was purchased from Phenomenex® Corp.

**Evaluation of CYP2C8 activity in human liver microsomes.** Human liver microsomes were pooled from 50 male and female donors, characterized by BD Gentest™, and then stored at 20 mg/ml in 250 mM sucrose at –70°C until use. The microsomes were thawed and then diluted with incubation buffer (100 mM potassium phosphate, 5 mM magnesium chloride, 1 mM EDTA). Assays were conducted in triplicate under optimized conditions (i.e., protein and substrate concentrations and incubation time) in a total incubation volume of 160 µl. Stock solutions of abiraterone acetate, abiraterone, abiraterone sulfate, and abiraterone sulfate-N-oxide were prepared at 10 or 30 mM in DMSO, serially diluted in DMSO, and then added to the microsomal suspension to yield eight final concentrations ranging from 0 to 30 or 60 µM, depending on the test compound. Because of the substantial CYP2C8 inhibition observed with abiraterone sulfate up to 30 µM, the assay was repeated at lower inhibitor concentrations (up to 5 µM) to obtain a more accurate IC₅₀ determination, defined as the inhibitor concentration producing a 50% reduction in maximal CYP2C8 enzyme activity. As a positive control, the CYP2C8 inhibitor quercetin was evaluated in triplicate at concentrations ranging from 0.03 to 30 µM. The probe substrate amodiaquine was added to the microsome suspension to yield a final concentration of 2 µM. The organic content in the final incubation mixture did not exceed 0.35%. For the time-dependent assay, gemfibrozil 1-O-β-glucuronide was evaluated as a time-dependent positive control.
After preincubation at 37°C for 10 min, the reactions to determine whether CYP2C8 activity was reversibly inhibited by abiraterone acetate, abiraterone, abiraterone sulfate, and abiraterone sulfate-N-oxide were initiated by addition of an NRS (40 µl), yielding final concentrations of 1.3 mM NADP⁺, 3.3 mM glucose-6-phosphate, and 0.4 U/ml glucose-6-phosphate dehydrogenase. After incubation for 15 min, the reactions were quenched by addition of 160 µl acetonitrile, and then transferred to a 2-ml Strata™ Impact protein precipitation 96-well plate containing 300 µl acetonitrile and 100 µl N-desethylamodiaquine-d₃ (1.5 µM) as internal standard. The samples were filtered, evaporated to dryness under nitrogen, and reconstituted in 250 µl of mobile phase (1:1 methanol:water containing 0.1% acetic acid). Time-dependent inhibition was assessed by incubating eight serially diluted concentrations (up to 15 µM) of the test compounds with and without NRS for 60 min and then transferring an aliquot to a secondary incubation containing 10 µM amodiaquine (5× Km) and NRS to assay residual CYP2C8 activity. After 15 min, samples were quenched and prepared as described for the reversible inhibition samples. Analysis by LC-MS/MS was carried out on a Shimadzu binary 10AD-vp liquid chromatography pump system with Leap CTC PAL autosampler coupled to a Sciex API 4000™ triple quadrupole mass spectrometer. The possibility of ion suppression of the analyte due to co-elution with the test compound was evaluated prior to injection of the incubated samples. The acquired data were processed using Analyst® version 1.5.1 (AB Sciex, Concord, ON, Canada) and the area ratios of metabolite and internal standard peaks were exported to Excel (Microsoft, Redmond, WA, USA) and SigmaPlot version 11 (Systat Software, Inc., San Jose, CA, USA) for nonlinear regression analysis.
The inhibitory effect of test compounds on CYP2C8-mediated amodiaquine metabolism was evaluated by determining the rate of formation of N-desethylamodiaquine as a function of inhibitor concentration, normalized to the maximal activity in the absence of inhibitor (i.e., vehicle control). IC\textsubscript{50} values for the inhibition of CYP2C8 enzyme activity was determined from semi-log plots of residual activity versus inhibitor concentration. In the time-dependent assay, separation between the plus and minus NADPH titration curves of percent residual CYP2C8 activity as a function of the molar ratio of inhibitor to CYP2C8 concentration would indicate the potential for time-dependent inhibition. In addition, the apparent partition ratio, representing the ratio of substrate molecules turned over per enzyme molecule inactivated, was determined from the extrapolated x-intercept of the initial linear portion of the titration curve.

**Evaluation of OATP1B1 activity in hepatocytes.** Cryopreserved human hepatocytes were thawed according to the manufacturer’s instructions. The hepatocytes were suspended at a concentration of 1×10\textsuperscript{6} cells/ml in Krebs-Henseleit buffer containing Hepes 12.5 mM, pH 7.4, and preincubated on a shaker at 37°C for at least 5 min. Test compound (6, 20, and 40 µM abiraterone; or its metabolites 6, 20, and 40 µM abiraterone sulfate; or 6, 20, and 60 µM abiraterone sulfate-N-oxide), positive reference inhibitors (100 µM rifampicin or 10 µM cyclosporin A), or solvent (DMSO, negative control) was added; and then, after an additional preincubation period, uptake was initiated by adding radiolabeled OATP1B1 substrate (E2-17G; final concentration 1 µM; 10 GBq/mmol) or \textsuperscript{3}H-repaglinide [final concentration 0.5 µM; 10 GBq/mmol]) to the hepatocyte suspension (final volume 2 ml). All incubations were stirred continuously.
Triplicate 200 µl samples were removed after 1 and 5 min, and transferred to Eppendorf 1.5 ml microtubes prefilled with 400 µl silicon/mineral oil (82:18, v/v) layered over 10% perchloric acid (250 µl) and immediately centrifuged (10,500 rpm) for 30 s in a swinging bucket centrifuge (Eppendorf 5417R). After centrifugation, the tubes were frozen on dry ice, the content cut at the oil interface, and both parts transferred into separate scintillation vials. To evaluate passive background uptake, negative control incubations were performed at 0 to 4°C, with triplicate samples removed after 1 and 5 min, and processed as described above. Protein concentrations in the incubation mixtures were quantified by bicinchoninic acid assay. The measured radioactivity was converted to picomoles uptake per 10^6 cells and per mg protein, and IC_{50} values calculated as the concentration producing 50% inhibition of the initial linear uptake rate of the probe substrate.

After correction for background radioactivity, measured radioactivity was converted to picomoles uptake per 10^6 cells and per mg protein, using Microsoft Excel. After plotting % residual hepatic uptake (compared with 100% residual activity in the solvent control) against the inhibitor concentration, IC_{50} values were calculated using a nonlinear regression program (Enzyme Kinetics 1.3 module of Sigmaplot 12.0, Systat Software, Inc., Port Richmond, CA).

**Clinical study. Study design and population.** A single-center, open-label, two-period, sequential-design study was conducted in healthy male subjects. An independent ethics committee approved the study protocol before any study-related procedures were started. The study was conducted in accordance with the ethical principles originating in the Declaration of Helsinki, and in compliance with Good Clinical Practices and applicable regulatory requirements. Eligible
subjects were males aged 18 to 55 years, with a body mass index of 18 to 30 kg/m², body weight ≥50 kg, and normal cardiac function. Subjects with a history of or current clinically significant medical illness, abnormal liver or renal function, or history of drug or alcohol abuse within the past 3 years were excluded. All subjects provided written informed consent before enrollment.

Subjects were admitted to the study center on day −1. After an overnight fast of at least 10 h, all subjects received pioglitazone 15 mg (1 × 15-mg tablet; TEVA, Castleford, UK) on day 1, and abiraterone acetate 1000 mg (4 × 250-mg tablets; Janssen-Cilag, Beerse, Belgium) followed 1 h later by pioglitazone on day 8. A one-sequence crossover design was applied to the study based on guidance from the US Food and Drug Administration (FDA) (Food and Drug Administration 2012). This proposed study design allows for a clean measurement of the pharmacokinetics of pioglitazone and its metabolites at baseline (i.e., control) from all subjects prior to coadministration with abiraterone acetate and minimizes unexpected carryover effect in case a significant interaction occurs. Study drugs were swallowed whole with 240 ml of noncarbonated water. A standardized breakfast with medium fat content was provided on both days after the pioglitazone dose, but no other food was to be consumed for 4 h. Throughout the study, prescription or nonprescription medications other than the study drugs were prohibited, except for acetaminophen.

**Pharmacokinetics.** Serial blood samples were collected predose and up to 72 h after each pioglitazone dose for determination of plasma concentrations of pioglitazone, M-III, and M-IV. Serial blood samples were also collected after administration of abiraterone acetate on day 8 for the determination of plasma abiraterone concentrations.
Plasma concentrations of abiraterone were determined using a validated method described previously (Marbury et al., 2014). In brief, a liquid-liquid extraction with t-butyl methyl ether followed by LC-MS/MS was carried out. All results were within the predefined acceptance criteria as described in guidance from the FDA (Food and Drug Administration 2001). The lower limit of quantification (LLOQ) was 0.2 ng/ml.

Pioglitazone plasma concentrations were determined using a validated method. Samples were subjected to protein precipitation with acetonitrile using a stable isotope-labeled internal standard. After centrifugation, the supernatant was injected into an LC-MS/MS system. A gradient of ammonium acetate (pH 2.5) with acetonitrile was run over an Xbridge C18 column. Detection was done with multiple reaction monitoring on an API4000, with a Turbo-Ionspray™ interface, operating in positive ion mode. The LLOQ was 0.5 ng/ml. All results were within the predefined acceptance criteria. Plasma concentrations of the M-III and M-IV metabolites of pioglitazone were determined using an LC-MS/MS assay. Samples were subjected to protein precipitation with acetonitrile using a stable isotope-labeled internal standard. After centrifugation, the supernatant was injected into an LC-MS/MS system. A gradient of ammonium formate (pH 2.5) with methanol was run over a Luna Phenylhexyl column. Detection was done with multiple reaction monitoring on an API4000, with a Turbo-Ionspray™ interface, operating in positive ion mode. The LLOQ for both metabolites was 0.5 ng/ml. All results were within the predefined acceptance criteria.
Pharmacokinetic parameters for pioglitazone, M-III, M-IV, and abiraterone were determined from the individual plasma concentration data and actual sampling times using noncompartmental analysis with validated WinNonlin software, version 6.3 (Pharsight Corp [Certara], Princeton, NJ, USA). The primary pharmacokinetic parameters of interest were the maximum observed plasma concentration (C_{max}), the area under the plasma concentration–time curve from time zero to the last observed quantifiable concentration (AUC_{last}), and the AUC from time zero to infinite time (AUC_{∞}). AUC_{last} was calculated by linear trapezoidal method. AUC_{∞} was calculated as the sum of AUC_{last} and C_{last}/λ_z, where C_{last} was the last observed quantifiable concentration.

**Safety.** Safety was monitored throughout the study and included adverse events, vital signs, clinical laboratory testing, 12-lead electrocardiograms, and physical examinations. Adverse events were coded according to the Medical Dictionary for Regulatory Activities (MedDRA) Version 16.0, and their severity graded according to the National Cancer Institute–Common Terminology Criteria for Adverse Events (NCI-CTCAE) Version 4.0.

**Statistics.** Using an intrasubject coefficient of variation of 22% for C_{max} and 19% for AUC based on published data on pioglitazone (Karim et al., 2007), a sample size of 12 subjects was considered sufficient for point estimates of the geometric mean ratios (GMRs) of C_{max} and AUC, with and without coadministration of abiraterone acetate, to fall within 85% and 117% and within 87% and 115% of their true values, respectively, with 90% confidence. A sample size of 16 subjects was planned, assuming a dropout rate of 25%.
Mean plasma concentration data and mean pharmacokinetic parameters were summarized descriptively. The statistical analysis included subjects who had pharmacokinetic parameter estimates for pioglitazone on both day 1 (pioglitazone alone) and day 8 (pioglitazone coadministered with abiraterone). The GMRs of the pharmacokinetic parameters ($C_{\text{max}}$, $AUC_{\text{last}}$, and $AUC_{\infty}$) of pioglitazone with and without coadministration of abiraterone acetate and the associated 90% confidence intervals (CIs) were constructed using least squares means and intrasubject coefficients of variation from a mixed-effects model of log-transformed pharmacokinetic parameters. A similar statistical analysis was done on the pharmacokinetic parameters of M-III and M-IV. All safety parameters were summarized descriptively.
RESULTS

Effect on CYP2C8 activity in human liver microsomes. Abiraterone acetate, abiraterone, and the major human metabolites of abiraterone inhibited the CYP2C8-mediated metabolism of amodiaquine to N-desethylamodiaquine in human liver microsomes in a concentration-dependent manner (Fig. 1). The rank order of potency for inhibiting CYP2C8 was abiraterone sulfate > abiraterone acetate ≈ abiraterone > abiraterone sulfate-N-oxide (Table 1). Only abiraterone sulfate had submicromolar IC₅₀ values for CYP2C8 inhibition (0.04 µM) using a concentration range of 0.005 to 5.0. In comparison, the respective IC₅₀ value for abiraterone sulfate-N-oxide was 5.4 µM. The IC₅₀ values for CYP2C8 inhibition for abiraterone acetate and abiraterone were 3.0 µM and 2.9 µM, respectively.

To determine whether CYP2C8 was inhibited in a time-dependent manner, the experiments were conducted in the presence and absence of an NRS during preincubation. Gemfibrozil 1-O-β-glucuronide was included as a time-dependent positive control inhibitor, and quercetin was included as a reversible negative control inhibitor. Gemfibrozil 1-O-β-glucuronide produced substantial inhibition of CYP2C8 activity in the presence of NADPH but minimal enzyme activity loss in the absence of NADPH; the apparent partition ratio (APR), which represents the ratio of substrate turnover per molecule of enzyme being inactivated, was 78, indicating strong and rapid time-dependent inhibition of CYP2C8. In cases of time-dependent inhibition, we defined APR values less than 100 as strong inhibition and values between 100 and 500 as moderate inhibition (Lim et al., 2005). Conversely, there was no NADPH-dependent loss of CYP2C8 activity with quercetin, abiraterone, abiraterone sulfate, or abiraterone sulfate N-oxide, indicating no time-dependent inhibition of CYP2C8 (Fig. 2), and therefore APR values were not
determined. The effects on CYP2C8 activity observed in the presence and absence of NADPH by these compounds reflected reversible inhibition. Since incubation times were short (e.g., 8–12 min for amodiaquine) the inhibitor/metabolite was presumed stable except for any NADPH-dependent turnover.

Effect on OATP1B1 activity in human hepatocytes. Based on the CYP2C8 inhibition results in human liver microsomes, a clinical drug-drug interaction study was planned to further evaluate the relevance of the in vitro findings. According to the literature, repaglinide is the highly sensitive CYP2C8 probe substrate (VandenBrink et al., 2011). However, because repaglinide is transported via OATP1B1 (Kalliokoski et al., 2008), the effect of abiraterone and its metabolites on OATP1B1-mediated uptake of $^3$H-E2-17G and $^3$H-repaglinide was first investigated in vitro using human hepatocytes. Cell viability was 82% in the presence of 0.25% DMSO vehicle, and was unaffected by addition of the test compounds. Abiraterone and its metabolites inhibited uptake of $^3$H-E2-17G and $^3$H-repaglinide when measured after incubations of 1 and 5 min (Fig. 3). At the lowest concentration tested, abiraterone 6 µM inhibited $^3$H-E2-17G uptake by 68.6% and $^3$H-repaglinide uptake by 58.5% at 5 min. When corrected for the passive component of total uptake, the inhibition by abiraterone 6 µM was 78% and 96%, respectively. Comparable results were found with abiraterone sulfate and abiraterone sulfate N-oxide (Fig. 3). The positive controls, cyclosporin A and rifampicin, also efficiently inhibited $^3$H-E2-17G and $^3$H-repaglinide uptake. The underlying cause of the increase uptake of $^3$H-repaglinide in the presence of abiraterone sulfate N-oxide 6 µM is unknown (Fig. 3B).
Effect on CYP2C8 activity in healthy volunteers. Because abiraterone and its metabolites were found to inhibit OATP1B1-mediated uptake of repaglinide in vitro, it was necessary to select another CYP2C8 probe substrate for the clinical drug-drug interaction study. Pioglitazone was selected due to its sensitivity to CYP2C8 inhibition, its demonstrated safety and tolerability in healthy volunteers, and the short washout period needed between doses. Moreover, a 15-mg dose of pioglitazone provides sufficient plasma drug levels for bioanalytical quantification through 48 h after dosing.

Sixteen healthy male subjects were enrolled. The study cohort had a median age of 46.5 years (range: 23–54 years), median weight of 81.3 kg (range: 60.6–101 kg), and median body mass index of 25.6 kg/m² (range: 20.3–28.4 kg/m²); all were white. Fifteen subjects completed the study as planned; one subject was discontinued prior to administration of study drugs on day 8 due to a positive screen for codeine.

Following oral administration of pioglitazone, mean plasma pioglitazone concentrations increased rapidly, with a median time to reach C\text{max} (t\text{max}) of 3.0 h when given alone and 4.0 h when given 1 h after abiraterone acetate (Fig. 4A). Thereafter, plasma pioglitazone concentrations declined in an apparent first-order manner, with a mean elimination half-life (t\text{1/2}) of 9.3 h when administered alone and 8.6 h when coadministered with abiraterone acetate. In general, the shapes of the plasma concentration–time curves were similar after both pioglitazone doses, although mean plasma pioglitazone concentrations appeared to be slightly higher at all time points when pioglitazone was coadministered with abiraterone acetate. The pharmacokinetic parameters for pioglitazone are shown in Table 3. Most subjects had higher
pioglitazone $C_{\text{max}}$, $AUC_{\text{last}}$, and $AUC_{\infty}$ values when pioglitazone was dosed 1 h after abiraterone acetate compared with when pioglitazone was administered alone (Fig. 5). Based on the GMRs in the statistical analysis, the $C_{\text{max}}$ of pioglitazone increased by 25% and the $AUC_{\text{last}}$ and $AUC_{\infty}$ increased by approximately 46% when pioglitazone was coadministered with abiraterone acetate.

M-III and M-IV appeared slowly in plasma, reaching peak concentrations after a median of 12 h when pioglitazone was administered alone and after a median of 24 h when pioglitazone was administered after abiraterone acetate (Figs. 4B and 4C). In general, the shapes of the M-III and M-IV concentration–time curves were similar after each pioglitazone dose, although mean plasma concentrations of both metabolites appeared to be slightly lower at all time points when pioglitazone was coadministered with abiraterone acetate. Based on the GMRs, the $C_{\text{max}}$ of M-III was reduced by 13% and the $AUC_{\text{last}}$ was reduced by 10% when pioglitazone was coadministered with abiraterone acetate; the $AUC_{\infty}$ was not affected by abiraterone acetate administration (Table 2). For M-IV, the $C_{\text{max}}$ was reduced by nearly 12%, the $AUC_{\text{last}}$ was reduced by 9.5%, and the $AUC_{\infty}$ was lowered by 8.5% when abiraterone acetate was administered before pioglitazone.

Following administration of abiraterone acetate on day 8, mean plasma abiraterone concentrations increased rapidly, with a median $t_{\text{max}}$ of 1.5 h (range: 1.5–3.0 h). Mean ± SD values for key pharmacokinetic parameters were 242 ± 123 ng/ml for $C_{\text{max}}$, 894 ± 373 h·ng/ml for $AUC_{\text{last}}$, and 908 ± 376 h·ng/ml for $AUC_{\infty}$. The apparent terminal elimination $t_{1/2}$ was 14.5 ± 3.1 h.
Treatment-emergent adverse events were reported by six subjects (38%) following pioglitazone alone and by two subjects (13%) following administration of pioglitazone after abiraterone acetate. All adverse events were mild in severity and resolved by the end of the study. Headache occurred in two subjects; all other adverse events were reported in one subject each. None was considered related to study treatment. There were no deaths, serious adverse events, or adverse events leading to discontinuation, or any clinically meaningful changes in other safety parameters.

**Discussion**

The results of the in vitro studies demonstrated that abiraterone acetate, abiraterone, and its sulfate and sulfate N-oxide metabolites were inhibitors of CYP2C8 activity in human liver microsomes. Of these, abiraterone sulfate exhibited the most potent CYP2C8 inhibitory activity. Although not compared in the same assay, the data suggest that abiraterone sulfate is at least 10-fold more potent than abiraterone and its prodrug as a CYP2C8 inhibitor. Abiraterone acetate and abiraterone sulfate were predicted to have Ki values \([\text{inhibition constant} = 0.5 \times IC_{50}]\) below 1 µM, which suggest high in vitro potency to cause in vivo drug interaction of at least 2-fold (Obach et al., 2005). In a previous study, \(C_{\text{max}}\) of abiraterone, abiraterone sulfate, and N-oxide abiraterone sulfate were reported to be approximately 0.37 µM, 25 µM, and 9.2 µM, respectively, following a single-dose administration of 1000 mg abiraterone acetate to healthy volunteers (Bernard et al., 2015). The calculated R values \((= 1 + [I]/Ki)\) were greater than 1.1 for abiraterone and its metabolites, thus pointing toward the need for a clinical study to assess the interaction further. Although the concentrations of abiraterone acetate in blood were below the LLOQ of the assay in the vast majority of subjects (Acharya et al., 2012; Acharya et al., 2013),
the concentration in the liver is unknown, and therefore in vitro testing was conducted. The inhibition of CYP2C8 by abiraterone and its metabolites was reversible, as no evidence of time-dependent inhibition was seen. Although not performed as a fully quantitative investigation for OATP1B1 in vitro inhibition, the outcome was that pioglitazone rather than repaglinide was selected for the clinical study designed to ascertain if the CYP2C8 inhibitory activity seen in vitro has clinical significance.

In the clinical study, the pharmacokinetic analysis showed that oral coadministration of abiraterone acetate increased the $C_{\text{max}}$ for pioglitazone by 25% and exposure to pioglitazone as measured by $\text{AUC}_{\text{last}}$ and $\text{AUC}_{\infty}$ by 46% compared with administration of a single 15-mg dose of pioglitazone alone. Consistent with the increased exposure to pioglitazone, the GMR calculations showed that the $C_{\text{max}}$ and $\text{AUC}_{\text{last}}$ of M-III (keto derivative) and M-IV (hydroxyl derivative) were reduced by 10 to 13% when pioglitazone was administered with versus without abiraterone acetate. The small decreases in metabolite concentrations suggest that their formation was minimally affected by CYP2C8 inhibition caused by abiraterone. While the enzymes involved in the metabolism of M-IV and M-III have not been identified, it has been reported that clearance of M-IV and M-III is inducible by rifampicin (Bernard et al., 2015; Jaakkola et al., 2006a), suggesting that CYP450s regulated by the pregnane X receptor gene may play a role (e.g., CYP3A4, CYP2C family). Considering the overall effect based on exposures to the total active compounds (sum of pioglitazone, M-III, and M-IV), it is probable that the net effect of abiraterone is a slight increase in the efficacy of pioglitazone by approximately 26% only. This magnitude of interaction is of little clinical relevance as it is comparable to that of ketoconazole, which is reported to increase the pioglitazone AUC by 34%, yet no dose adjustment or caution is
necessary for pioglitazone when coadministered with ketoconazole (ACTOS [US prescribing information] 1999). Based on the extent of increase in pioglitazone exposure in this study, we conclude that administration of abiraterone acetate leads to mild/weak inhibition of CYP2C8, in accordance with regulatory guidelines from the European Medicines Agency (EMEA) and the US Food and Drug Administration (FDA) (European Medicines Agency Committee for Human Medicinal Products (CHMP) 2012; US Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research 2012). Moreover, the safety and tolerability assessment revealed no clinically relevant abnormalities when abiraterone acetate was coadministered with pioglitazone, consistent with prior experience with abiraterone in other clinical studies in healthy adult males.

In this clinical interaction study, a standardized breakfast with medium fat was given 1 h after the abiraterone acetate dose to obtain pharmacokinetic exposure to abiraterone similar to that observed previously in a multidose study conducted in cancer patients (COU-AA-006) (Tagawa et al., 2016). In that study, food intake followed the approved dosing and administration recommendation, with a meal allowed 2 h before or 1 h after abiraterone acetate dosing. The pharmacokinetic analysis showed an arithmetic mean $C_{\text{max}}$ of 207 ng/ml and an arithmetic mean AUC from time 0 to 24 h of 965 h·ng/ml, which compare favorably with the $C_{\text{max}}$ of 242 ng/ml and AUC$_{\infty}$ of 908 ng·h/ml found in the present single-dose study.

Although the in vitro data suggest that abiraterone and its metabolites are potent CYP2C8 inhibitors, the clinical data are inconsistent with this finding. The extrapolation of in vitro data to predict in vivo drug-drug interactions can be affected by multiple biochemical and biophysical
factors, including the biochemical assay environment and substrate-dependent effects (Wienkers and Heath, 2005). Abiraterone is highly bound (>99%) to plasma proteins (Zytiga [prescribing information] 2015); abiraterone sulfate and abiraterone sulfate N-oxide were also found to be highly protein-bound in healthy subjects. The free fraction of these metabolites was <0.15% and 0.23%, respectively, and did not change when assessed in the presence of abiraterone (<0.15% and 0.22%, respectively) (data on file, Janssen Research & Development). Further, the fractions unbound in the CYP2C8 microsomal medium were 0.047, 0.42, and 0.901 \( \mu \text{M} \) for abiraterone, abiraterone sulfate, and abiraterone sulfate N-oxide, respectively, which resulted in IC\(_{50} \) values corrected for binding in the liver microsomes of 0.06 to 0.07 \( \mu \text{M} \) for abiraterone, 0.063 to 0.018 \( \mu \text{M} \) for abiraterone sulfate, and 5.3 to 4.87 \( \mu \text{M} \) for abiraterone sulfate N-oxide. Comparing these values with the unbound C\(_{\text{max}} \) concentrations (0.0005 \( \mu \text{M} \) for abiraterone, 0.037 \( \mu \text{M} \) for abiraterone sulfate, and 0.055 \( \mu \text{M} \) for N-oxide abiraterone sulfate), only free plasma concentrations of abiraterone sulfate were in the order of magnitude of the IC\(_{50} \) (data on file, Janssen Research & Development). Applying the mechanistic static model (European Medicines Agency Committee for Human Medicinal Products (CHMP) 2012), using the unbound C\(_{\text{max}} \) for abiraterone sulfate and a fraction metabolized of 0.78 for pioglitazone (Xiao et al., 2015), resulted in an AUC ratio of 2.7, which was still above the upper limit (1.7) of the measured increase in AUC ratio of pioglitazone, suggesting that the unbound C\(_{\text{max}} \) overestimates the interaction in the equation. The static drug-drug interaction equation assumes that the maximal concentrations of abiraterone and its metabolites are constant over time, whereas in reality abiraterone sulfate (currently the major driver of the CYP2C8 drug-drug interaction) has a plasma half-life of 2.47 h. This means that at 4 h (t\(_{\text{max}} \)) after dosing, mild CYP2C8 inhibition
occurs, but at 24 h after dosing, in reality, no CYP2C8 inhibition of the sulfate metabolite is expected to occur.

It is unlikely that the use of amodiaquine for the in vitro studies and pioglitazone for the in vivo studies would account for the observed differences in potency of CYP2C8 inhibition as both have been reported to have comparable IC$_{50}$ values in the presence of CYP2C8 inhibitors (Albassam et al., 2015; Jaakkola et al., 2006c). These results are not unique to abiraterone acetate. Similar findings were observed with the leukotriene antagonists montelukast and zafirlukast, which showed strong inhibition of CYP2C8 in vitro (IC$_{50}$ = 0.51 µM and 1.0 µM, respectively) (Jaakkola et al., 2006c), but did not significantly affect the pharmacokinetics of pioglitazone or its metabolites in vivo (Jaakkola et al., 2006b). The results of our studies and those by Jaakkola et al. highlight the impact of relevant pharmacokinetic properties and plasma protein binding data on the outcome of in vitro–in vivo interaction predictions.

Abiraterone and its metabolites were found to inhibit the hepatic uptake transporter OATP1B1 in vitro. For abiraterone, the in vivo OATP1B1 inhibition potential was calculated according to the FDA and EMEA drug-drug interaction guidance (European Medicines Agency Committee for Human Medicinal Products (CHMP) 2012; US Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research 2012). Based on the calculated in vivo inhibition potential, any interaction for abiraterone is considered negligible as R was below 1.25 (Food and Drug Administration 2012) or $K_i$ was greater than 50*C$_{max}$(European Medicines Agency Committee for Human Medicinal Products (CHMP) 2012). The guidelines do not mention calculation of the in vivo interaction potential for metabolites.
However, there are no clinical data available to confirm the in vitro transporter based interaction for the metabolites of abiraterone acetate.

In summary, abiraterone acetate and abiraterone and its major human metabolites exhibited considerable inhibition of CYP2C8 in human liver microsomes in vitro. However, administration of abiraterone acetate to healthy male subjects had only weak effects on the pharmacokinetics of the CYP2C8 probe substrate pioglitazone. Clinicians should consider the therapeutic index of CYP2C8 substrates when administering abiraterone acetate and follow approved product dosing recommendations.
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Authorship Contributions.

*Participated in research design:* Chien, Gonzalez, Jiao, Sensenhauser, Snoeys, and Wynant.

*Conducted experiments:* Gonzalez, Sensenhauser, Smit, and Wynant.

*Contributed new reagents or analytic tools:* Stieltjens.

*Performed data analysis:* Bernard, Chien, Jiao, Sensenhauser, and Smit.

*Wrote or contributed to the writing of the manuscript:* Monbaliu, Gonzalez, Bernard, Jiao, Sensenhauser, Snoeys, Stieltjes, Wynant, Smit, and Chien.


Footnote

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**Figure Legends**

Fig. 1. Concentration-response curves for CYP2C8 inhibition by abiraterone acetate, abiraterone, its metabolites, and the positive control quercetin in human liver microsomes. Shown are the results from assay 1 (A), assay 2 (B), assay 3 (C), and assay 4 (D). Each point is the mean of three replicate determinations. Outliers had poor solubility as evidenced by visible precipitation.

Fig. 2. Evaluation of time-dependent inhibition of CYP2C8 in human liver microsomes by incubation in the presence or absence of an NADPH-regenerating system.

Fig. 3. Effect of abiraterone and its metabolites on OATP1B1-mediated uptake of $^3$H-E2-17G and $^3$H-repaglinide in human hepatocytes after 1 min (A) and 5 min (B) of incubation.

Fig. 4. Log-linear mean plasma pioglitazone (A), M-III (B), and M-IV (C) concentration–time curves following a single 15-mg oral dose of pioglitazone administered alone or 1 h after abiraterone acetate.

Fig. 5. Individual and mean pharmacokinetic parameters for pioglitazone following single 15-mg oral dose of pioglitazone administered alone or 1 h after abiraterone acetate.
TABLE 1

*Effect of abiraterone acetate, abiraterone, and its metabolites on CYP2C8 activity in human liver microsomes*

<table>
<thead>
<tr>
<th>Assay</th>
<th>Test compound</th>
<th>Concentration range (µM)</th>
<th>IC₅₀ (µM)</th>
<th>Maximum % inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay 1</td>
<td>Abiraterone acetate</td>
<td>0.03–30.0</td>
<td>3.0</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Abiraterone</td>
<td>0.03–30.0</td>
<td>2.9</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>0.03–30.0</td>
<td>1.3</td>
<td>96</td>
</tr>
<tr>
<td>Assay 2</td>
<td>Abiraterone sulfate</td>
<td>0.005–5.0</td>
<td>0.044</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Abiraterone sulfate N-oxide</td>
<td>0.03–30.0</td>
<td>5.4</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>0.03–30.0</td>
<td>3.3</td>
<td>95</td>
</tr>
</tbody>
</table>
TABLE 2

Pharmacokinetic parameters of pioglitazone and its M-III and M-IV metabolites following administration of pioglitazone 15 mg alone or 1 h after abiraterone acetate

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pioglitazone alone (n = 16)</th>
<th>Pioglitazone + AA (n = 15)</th>
<th>GMR (90% CI) (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pioglitazone</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>338 (120)</td>
<td>415 (124)</td>
<td>125 (99.9–156)</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)$^a$</td>
<td>3.0 (0.5–4.0)</td>
<td>4.0 (0.5–4.0)</td>
<td>—</td>
</tr>
<tr>
<td>$AUC_{\text{last}}$ (h·ng/ml)</td>
<td>4440 (1220)</td>
<td>6330 (1330)</td>
<td>146 (126–171)</td>
</tr>
<tr>
<td>$AUC_{\infty}$ (h·ng/ml)</td>
<td>4490 (1220)</td>
<td>6380 (1330)</td>
<td>146 (126–169)</td>
</tr>
<tr>
<td>CL/F (L/h)</td>
<td>3.55 (0.866)</td>
<td>2.46 (0.599)</td>
<td>—</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>9.30 (2.52)</td>
<td>8.56 (2.30)</td>
<td>—</td>
</tr>
<tr>
<td><strong>M-III</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>72.5 (23.6)</td>
<td>61.7 (16.4)</td>
<td>86.6 (70.8–106)</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)$^a$</td>
<td>12.0 (6.0–36.0)</td>
<td>24.0 (12.0–36.0)</td>
<td>—</td>
</tr>
<tr>
<td>$AUC_{\text{last}}$ (h·ng/ml)</td>
<td>3110 (1090)</td>
<td>2710 (714)</td>
<td>89.9 (77.5–104)</td>
</tr>
<tr>
<td>$AUC_{\infty}$ (h·ng/ml)</td>
<td>3560 (1420)$^b$</td>
<td>3110 (821)$^c$</td>
<td>100 (85.8–117)$^e$</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>20.6 (3.00)$^b$</td>
<td>21.8 (3.11)$^c$</td>
<td>—</td>
</tr>
<tr>
<td>M/P $C_{\text{max}}$ ratio</td>
<td>0.21 (0.03)</td>
<td>0.15 (0.03)</td>
<td>—</td>
</tr>
<tr>
<td>M/P $AUC_{\text{last}}$ ratio</td>
<td>0.67 (0.12)</td>
<td>0.41 (0.09)</td>
<td>—</td>
</tr>
<tr>
<td>M/P $AUC_{\infty}$ ratio</td>
<td>0.75 (0.14)$^b$</td>
<td>0.48 (0.10)$^c$</td>
<td>—</td>
</tr>
<tr>
<td><strong>M-IV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>168 (62.8)</td>
<td>145 (36.7)</td>
<td>88.14 (74.7–104)</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value 1 (Range)</td>
<td>Value 2 (Range)</td>
<td>Value 3</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)$^a$</td>
<td>12.0 (8.0–24.0)</td>
<td>24.0 (12.0–36.0)</td>
<td>—</td>
</tr>
<tr>
<td>$\text{AUC}_{\text{last}}$ (h·ng/ml)</td>
<td>7580 (2321)</td>
<td>6720 (1450)</td>
<td>90.5 (79.8–103)</td>
</tr>
<tr>
<td>$\text{AUC}_x$ (h·ng/ml)</td>
<td>8380 (2410)$^d$</td>
<td>7620 (2030)$^c$</td>
<td>91.5 (82.0–102)$^e$</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>20.7 (4.10)$^d$</td>
<td>19.6 (2.68)$^c$</td>
<td>—</td>
</tr>
<tr>
<td>M/P $C_{\text{max}}$ ratio</td>
<td>0.48 (0.09)</td>
<td>0.34 (0.05)</td>
<td>—</td>
</tr>
<tr>
<td>M/P $\text{AUC}_{\text{last}}$ ratio</td>
<td>1.67 (0.42)</td>
<td>1.03 (0.16)</td>
<td>—</td>
</tr>
<tr>
<td>M/P $\text{AUC}_x$ ratio</td>
<td>1.97 (0.49)$^d$</td>
<td>1.20 (0.25)$^c$</td>
<td>—</td>
</tr>
</tbody>
</table>

CL/F, Apparent total clearance of the drug from plasma after oral administration; M/P, metabolite-to-parent ratio after correction for the difference in their molecular weight.

$^a$All pharmacokinetic parameters are reported as the mean (SD) except for $t_{\text{max}}$, for which the median (range) is reported.

$^b$For $\text{AUC}_x$, $n = 13$ due to one subject having a negative slope (lambda z not assessable) and two subjects having unacceptably high variability in the terminal phase.

$^c$For $\text{AUC}_x$, $n = 9$ due to one subject having a negative slope (lambda z not assessable) and five subjects having unacceptably high variability in the terminal phase.

$^d$For $\text{AUC}_x$, $n = 11$ due to five subjects having unacceptably high variability in the terminal phase.

$^e$For geometric mean ratio of M-III and M-IV $\text{AUC}_x$, $n = 8$. 
Figure 1

A

Quercetin ($\mu$M)

IC$_{50}$ = 1.3 $\mu$M

IC$_{50}$ = 3.0 $\mu$M

IC$_{50}$ = 2.9 $\mu$M

% Activity Remaining

0.1 1 10 100

0 20 40 60 80 100 120

AA ($\mu$M)

ABI ($\mu$M)

Outlier due to poor solubility

B

Quercetin ($\mu$M)

IC$_{50}$ = 3.3 $\mu$M

IC$_{50}$ = 0.044 $\mu$M

IC$_{50}$ = 5.4 $\mu$M

% Activity Remaining

0.1 1 10

0 20 40 60 80 100 120

ABI-S ($\mu$M)

ABI-SNO ($\mu$M)
Figure 3

A

Hepatic uptake (pmol/10^6 cells)

3H-E2-17G

3H-repaglinide

Vehicle
ABI 6 µM
ABI 20 µM
ABI 40 µM
ABI-S 6 µM
ABI-S 20 µM
ABI-S 40 µM
ABI-SNO 6 µM
ABI-SNO 20 µM
ABI-SNO 60 µM
Rifampicin 100 µM
Cyclosporin A

B

Hepatic uptake (pmol/10^6 cells)

3H-E2-17G

3H-repaglinide

Vehicle
ABI 6 µM
ABI 20 µM
ABI 40 µM
ABI-S 6 µM
ABI-S 20 µM
ABI-S 40 µM
ABI-SNO 6 µM
ABI-SNO 20 µM
ABI-SNO 60 µM
Rifampicin 100 µM
Cyclosporin A
Figure 4

A

Pioglitazone (ng/mL Plasma)

Time (h)

Pioglitazone (n = 16)
Pioglitazone + AA (n = 15)

B

Pioglitazone M-III (ng/mL Plasma)

Time (h)

Pioglitazone (n = 16)
Pioglitazone + AA (n = 15)

C

Pioglitazone M-IV (ng/mL Plasma)

Time (h)

Pioglitazone (n = 16)
Pioglitazone + AA (n = 15)
Figure 5

A

Pioglitazone C_{max} (ng/mL)

Pioglitazone Pioglitazone + AA

Treatment

B

Pioglitazone AUC_{0-4h} (h-ng/mL)

Pioglitazone Pioglitazone + AA

Treatment

C

Pioglitazone AUC_{0-4h} (h-ng/mL)

Pioglitazone Pioglitazone + AA

Treatment