Short Communication

Bedaquiline Metabolism: Enzymes and Novel Metabolites^S

Received November 15, 2013; accepted February 10, 2014

ABSTRACT

Bedaquiline is a recently approved drug for the treatment of multidrug-resistant tuberculosis. Adverse cardiac and hepatic drug reactions to bedaquiline have been noted in clinical practice. The current study investigated bedaquiline metabolism in human hepatocytes using a metabolomic approach. Bedaquiline *N*-demethylation via CYP3A4 was confirmed as the major pathway in bedaquiline metabolism. In addition to CYP3A4, we found that both CYP2C8 and

CYP2C19 contributed to bedaquiline *N*-demethylation. The K_m values of CYP2C8, CYP2C19, and CYP3A4 in bedaquiline *N*-demethylation were 13.1, 21.3, and 8.5 μM , respectively. We also identified a novel metabolic pathway of bedaquiline that produced an aldehyde intermediate. In summary, this study extended our knowledge of bedaquiline metabolism, which can be applied to predict and prevent drug–drug interactions and adverse drug reactions associated with bedaquiline.

Introduction

Multidrug-resistant tuberculosis (MDR-TB) is defined as tuberculosis (TB) with resistance to at least isoniazid and rifampicin, the two most powerful first-line anti-TB drugs (Chang and Yew, 2013). There were approximately 0.31 million incident cases of MDR-TB among patients with TB in 2011. Treatment of MDR-TB requires second-line anti-TB agents given over a period of 18–24 months (Mukherjee et al., 2004). Significant side effects and a high patient death rate were observed during long-term treatment against MDR-TB (World Health Organization, 2013). Thus, there is an urgent need to develop a shorter and less toxic regimen to reduce the side effects and mortality associated with MDR-TB chemotherapy.

Bedaquiline (BDQ) was recently approved by the U.S. Food and Drug Administration (FDA, 2012b) for the treatment of MDR-TB. BDQ is a diarylquinoline with a novel mechanism of action specifically inhibiting mycobacterial ATP synthase (Cole and Alzari, 2005). In a randomized phase II placebo-controlled clinical trial among patients with MDR-TB, 48% of patients who received BDQ in combination with a standard MDR regimen showed a conversion to negative culture in 8 weeks compared with 9% in the placebo group that received the standard MDR regimen (Diacon et al., 2009). After 24 weeks, the proportion of patients that reached treatment success was 78.8% in the BDQ group and 57.6% in the placebo group (Diacon et al., 2012). However, BDQ carries a boxed warning indicating that this drug can cause QT prolongation, which could lead to an abnormal and potentially fatal heart rhythm (FDA, 2012a). In addition, the rate of drug-related hepatic disorders in the BDQ group (8.8%) was higher than that in the placebo group (1.9%) (FDA, 2012a).

This research was supported in part by the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases [Grant R01-DK090305]; and the National Institutes of Health National Institute of Allergy and Infectious Diseases [Grant R56-AI095425].

dx.doi.org/10.1124/dmd.113.056119.

^SThis article has supplemental material available at dmd.aspetjournals.org.

Illustration of BDQ metabolism will provide valuable information to predict and prevent drug–drug interactions and adverse drug reactions associated with BDQ. CYP3A4 is involved in BDQ metabolism, leading to the formation of a dominant but less active *N*-desmethyl metabolite (Janssen Pharmaceutical Companies, 2012). BDQ and its metabolites, including *N*-desmethyl BDQ, *N*-didesmethyl BDQ, and two hydroxyl metabolites, are mainly excreted via feces (Cuycckens et al., 2008; Meermann et al., 2012). However, limited information is available for bioactivation pathways of BDQ that may be related to BDQ toxicity. In addition, it is unknown whether CYP3A4 is the only cytochrome P450 (P450) that contributes to BDQ metabolism. The current study was designed to explore novel metabolic pathways of BDQ metabolism using a metabolomic approach. The involvement of P450s in BDQ *N*-demethylation, the dominant pathway in BDQ metabolism, was also investigated using recombinant P450s. We identified a novel pathway in BDQ metabolism that produces an aldehyde intermediate. We also found that CYP2C8 and CYP2C19, together with CYP3A4, are involved in BDQ *N*-demethylation.

Materials and Methods

Chemicals. BDQ was purchased from Adooq Bioscience (Irvine, CA). Hepatocyte maintenance medium, dexamethasone, and insulin were obtained from Lonza (Walkersville, MD). Penicillin G/streptomycin and amphotericin B were obtained from Gibco Laboratories (Grand Island, NY). Type I (rat-tail) collagen was purchased from BD Biosciences (Bedford, MA). The recombinant human P450s and human liver microsomes were purchased from XenoTech, LLC (Lenexa, KS). Formic acid, β -nicotinamide-adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate, dimethylsulfoxide, ethyl acetate, and methoxylamine (MeONH₂) were obtained from Sigma-Aldrich (St. Louis, MO).

BDQ Metabolism in Human Hepatocytes. Fresh isolated human hepatocytes were plated at a cell density of 0.5×10^6 cells/well in 12-well plates previously coated with 0.2 mg/ml type I collagen. The isolated hepatocytes (>80% viability) were maintained in human maintenance medium supplemented with 100 nM dexamethasone, 100 nM insulin, 100 U/ml

ABBREVIATIONS: BDQ, bedaquiline; FDA, U.S. Food and Drug Administration; *m/z*, mass-to-charge ratio; MDR-TB, multidrug-resistant tuberculosis; MeONH₂, methoxylamine; P450, cytochrome P450; TB, tuberculosis; UPLC-QTOFMS, ultra-performance liquid chromatography coupled to time-of-flight mass spectrometry.

penicillin G, 100 $\mu\text{g/ml}$ streptomycin, and 10% bovine calf serum and kept at 37°C in a humidified incubator with 95% air/5% CO_2 . The hepatocytes were allowed to attach to the plate for 4 hours and the medium was then replaced with serum-free human maintenance medium. For the metabolism study, human hepatocytes were incubated with 30 μM BDQ for 18 hours. The medium was collected and hepatocytes were lysed by adding 200 μl acetonitrile/ H_2O (1:1, v/v). Both medium and hepatocyte lysates were extracted by adding a 4-fold volume of ethyl acetate. The mixture was then vortexed for 1 minute and centrifuged at 15,000g for 10 minutes. The organic phase was transferred to a glass tube and dried under a gentle stream of nitrogen. The residue was reconstituted by 100 μl acetonitrile/ H_2O (1:1, v/v). Five microliters of the solution was injected onto a system combining ultra-performance liquid chromatography coupled to time-of-flight mass spectrometry (UPLC-QTOFMS) for metabolite analysis.

BDQ Metabolism in Recombinant P450s and Human Liver Microsomes. Incubations were carried out in 1 $\times$ phosphate-buffered saline (pH 7.4), containing cDNA-expressed P450 isozymes and 30 μM BDQ with or without 5 mM of the aldehyde trapping reagent MeONH_2 in a final volume of 190 μl . The reactions were initiated by adding 10 μl of 20 mM β -nicotinamide-adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate and continued for 20 minutes with gentle shaking. The kinetics of *N*-desmethyl BDQ formation by cDNA-expressed P450s were determined by incubating BDQ at different concentrations up to 100 μM . Incubations were also carried out in human liver

microsomes and in the presence of MeONH_2 (5 mM) to trap aldehyde metabolites of BDQ.

UPLC-QTOFMS Analysis. Chromatographic separation of BDQ metabolites was performed on an Acquity UPLC BEH C18 column (2.1 $\times$ 100 mm, 1.7 μm ; Waters, Milford, MA). The flow rate of the mobile phase was 0.40 ml/min and the column temperature was maintained at 50°C. QTOFMS was operated in positive mode with electrospray ionization. The source and desolvation temperatures were set at 120°C and 350°C, respectively. Nitrogen was applied as the cone gas (10 l/h) and desolvation gas (700 l/h). Argon was applied as the collision gas. The capillary and cone voltages were set at 3.5 kV and 35 V. QTOFMS was calibrated with sodium formate and monitored by the intermittent injection of lock mass leucine enkephalin in real time. Screening and identification of BDQ metabolites were performed by using MarkerLynx software (Waters) based on accurate mass measurement (mass errors less than 10 ppm). Product ion scans with collision energy ramping from 15 to 45 V were used for structural elucidations of BDQ and its metabolites.

Data Analysis. Mass spectra were acquired by MassLynx 4.1 (Waters) in centroid format from m/z 50 to 1000. Centroid and integrated mass chromatographic data were processed by MarkerLynx software to generate a multivariate data matrix. These data were imported to EZinfo software (Umetrics, San Jose, CA) for orthogonal partial least-squares discriminant analysis on Pareto-scaled data. The corresponding data matrices were then exported into SIMCA-P+ (version 13; Umetrics) for multivariate data analysis.

Fig. 1. BDQ *N*-dealkylation and formation of an aldehyde and a carboxylic acid metabolite. (A–C) Identification of a carboxylic acid metabolite of BDQ (M6) in human hepatocytes. (A) Extracted ion chromatograph of M6. (B and C) MS/MS spectrum of M6 at m/z 542 and 544. (D–F) Trapping of the aldehyde (M5) intermediate in BDQ metabolism in human liver microsomes. MeONH_2 was used as a trapping agent. (D) Extracted ion chromatograph of M5-oxime. (E and F) MS/MS spectrum of M5-oxime at m/z 555 and 557. (G) Proposed mechanism for the formation of aldehyde (M5) and carboxylic metabolite (M6) in BDQ metabolism.

Results and Discussion

Profiling of BDQ Metabolites in Human Hepatocytes. Metabolomics has proved to be a powerful tool for profiling drug metabolism and bioactivation pathways (Ma et al., 2008; Li et al., 2011). In the current study, an UPLC-QTOFMS-based metabolomic approach was used to profile BDQ metabolism. The results of chemometric analysis on the ions produced by the UPLC-QTOFMS assay of control and BDQ-treated human hepatocytes are shown in Supplemental Fig. 1. The orthogonal partial least-squares discriminant analysis revealed two clearly separated clusters corresponding to the control and BDQ-treated groups (Supplemental Fig. 1A). The corresponding S-plot displays ions contributing to the group separation (Supplemental Fig. 1B). The top-ranking ions were identified as BDQ metabolites (Fig. 1 and Supplemental Figs. 3 and 4) based on mass defect shifts and bromide isotopic patterns. Overall, eight metabolites were identified in human hepatocytes, including six previously reported metabolites (M1, M2, M3, M4, M7 and M8) and two novel metabolites (M5 and M6). All BDQ metabolites identified in human hepatocytes were also found in the feces of mice treated with BDQ (data not shown). The BDQ metabolites are summarized in Supplemental Table 1.

Identification of Novel BDQ Metabolites. M6 was found as a novel BDQ metabolite during metabolomic analysis (Supplemental Fig. 1). The retention time of M6 was 8.90 minutes with protonated ions $[M + H]^+$ at a mass-to-charge ratio (m/z) of 542 and 544 in a nearly 1:1 ratio because of the $^{79}\text{Br}/^{81}\text{Br}$ isotopic pattern (Fig. 1). The MS/MS spectrum of M6 showed major fragment ions at m/z 327/329, 310/312, 236/238, and 91 (Fig. 1, B and C). Compared with the MS/MS spectrum of BDQ (Supplemental Fig. 2), the absence of ions at m/z 58, 229 suggest that the dimethylamino moiety has been removed. M6 was proposed as a carboxylic acid metabolite (Fig. 1A). In theory, M6 is an oxidized product of an aldehyde, which can be generated in *N*-dealkylation of BDQ. The aldehyde intermediate M5 was confirmed in the incubations with human liver microsomes. MeONH₂ was used as a trapping agent that produced M5-oxime as a stable adduct (Fig. 1D). M5-oxime was eluted at 9.52 minutes with protonated ions $[M + H]^+$ at m/z 555 and 557. Compared with fragment ions of M6 (Fig. 1, B and C), these same ions were observed in MS/MS analysis of M5-oxime, including m/z 327/329, 310/312, 236/238, and 91 (Fig. 1, E and F). These results suggest that BDQ *N*-dealkylation occurred and produced an aldehyde (M5) (Fig. 1G). Further studies demonstrated that M5 formation is primarily mediated by CYP3A4 (Supplemental Fig. 5). In general, production of an aldehyde is considered as an unwanted pathway in drug metabolism because aldehydes are more reactive and they may lead to toxicity (Uchida, 2000; O'Brien et al., 2005). Thus, this study provides a clue to investigate adverse drug reactions associated with BDQ in relation to BDQ *N*-dealkylation.

Role of P450s in BDQ *N*-Demethylation. When BDQ is metabolized, *N*-demethylation is the major metabolic pathway and *N*-desmethyl BDQ (M1) is the main circulating metabolite (FDA, 2012a). Compared with BDQ, M1 has a 3- to 6-fold lower antimycobacterial activity. However, M1 is more cytotoxic and is a stronger inducer of phospholipidosis than BDQ (Mesens et al., 2007). The formation of M1 is mediated primarily by CYP3A4 (Janssen Pharmaceutical Companies, 2012). We found that CYP2C8 and CYP2C19 are also involved in BDQ *N*-demethylation (Fig. 2A). The K_m values of CYP2C8, CYP2C19, and CYP3A4 in M1 formation were 13.1, 21.3, and 8.5 μM , respectively (Fig. 2B). These results indicate that CYP3A4 is not the only enzyme in BDQ *N*-demethylation. Thus, when patients receive BDQ together with drugs that are CYP3A4 inhibitors, BDQ *N*-demethylation may not be completely blocked, because CYP2C8 and CYP2C19 also contribute to BDQ *N*-demethylation. However,

Fig. 2. Role of P450s in BDQ *N*-demethylation (M1). cDNA-expressed P450s were used to determine the contribution of individual P450s in M1 formation. The incubation conditions were detailed in the experimental procedures and M1 was determined by UPLC-QTOFMS. (A) Relative contribution of P450s in M1 formation. The data are expressed as the mean $\pm$ S.D. ($n = 3$). The data in the CYP3A4 group were set as 100%. (B) Enzymatic kinetics of CYP2C8, CYP2C19, and CYP3A4 in M1 formation.

when patients receive BDQ together with drugs that are CYP3A4 inducers, such as ligands of the pregnane X receptor, BDQ *N*-demethylation may be extremely accelerated because activation of the pregnane X receptor induces the expression of CYP2C8, CYP2C19, and CYP3A4 (Gerbal-Chaloin et al., 2001; Luo et al., 2002).

In summary, BDQ metabolism was investigated in human hepatocytes by using a metabolomics approach. BDQ *N*-dealkylation was identified as a novel metabolic pathway of BDQ that produces an aldehyde intermediate. CYP2C8 and CYP2C19, together with CYP3A4, were involved in BDQ *N*-demethylation. These results can be applied to predict and prevent drug-drug interactions and adverse drug reactions associated with BDQ.

Center for Pharmacogenetics,
Department of Pharmaceutical
Sciences, School of Pharmacy,
University of Pittsburgh, Pittsburgh,
Pennsylvania (K.L., J.L., S.L., W.X.,
X.M.); and Department of
Pharmacology, Toxicology, and
Therapeutics, University of Kansas
Medical Center, Kansas City,
Kansas (F.L., K.D.)

KE LIU
FENG LI
JIE LU
SHINLAN LIU
KENNETH DORKO
WEN XIE
XIAOCHAO MA

Authorship Contributions

Participated in research design: K. Liu, Li, Ma.
Conducted experiments: K. Liu, Lu, S. Liu.

Contributed new reagents or analytic tools: K. Liu, Li, Dorko, Xie.

Performed data analysis: K. Liu, Ma.

Wrote or contributed to the writing of manuscript: K. Liu, Ma.

References

- Chang KC and Yew WW (2013) Management of difficult multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis: update 2012. *Respirology* **18**:8–21.
- Cole ST and Alzari PM (2005) Microbiology. TB—a new target, a new drug. *Science* **307**: 214–215.
- Cuyckens F, Balcaen LI, De Wolf K, De Samber B, Van Looveren C, Hurkmans R, and Vanhaecke F (2008) Use of the bromine isotope ratio in HPLC-ICP-MS and HPLC-ESI-MS analysis of a new drug in development. *Anal Bioanal Chem* **390**:1717–1729.
- Diacon AH, Donald PR, Pym A, Grobusch M, Patientia RF, Mahanyele R, Bantubani N, Narasimooloo R, De Marez T, and van Heeswijk R, et al. (2012) Randomized pilot trial of eight weeks of bedaquiline (TMC207) treatment for multidrug-resistant tuberculosis: long-term outcome, tolerability, and effect on emergence of drug resistance. *Antimicrob Agents Chemother* **56**:3271–3276.
- Diacon AH, Pym A, Grobusch M, Patientia R, Rustomjee R, Page-Shipp L, Pistorius C, Krause R, Bogoshi M, and Churchyard G, et al. (2009) The diarylquinoline TMC207 for multidrug-resistant tuberculosis. *N Engl J Med* **360**:2397–2405.
- FDA (2012a) *Briefing Package: NDA 204-384: Sirturo*, Division of Anti-Infective Products, Office of Antimicrobial Products, US Food and Drug Administration Center for Drug Evaluation, Silver Spring, MD.
- FDA (Press release, December 31, 2012b) *FDA Approves First Drug to Treat Multidrug-Resistant Tuberculosis*. US Food and Drug Administration, Silver Spring, MD.
- Gerbal-Chaloin S, Pascussi JM, Pichard-Garcia L, Daujat M, Waechter F, Fabre JM, Carrère N, and Maurel P (2001) Induction of CYP2C genes in human hepatocytes in primary culture. *Drug Metab Dispos* **29**:242–251.
- Janssen Pharmaceutical Companies (2012) *Briefing Document, TMC207 (Bedaquiline), Treatment of Patients with MDR-TB, NDA 204-384*, Janssen Pharmaceutical Companies, Titusville, NJ.
- Li F, Lu J, Wang L, and Ma X (2011) CYP3A-mediated generation of aldehyde and hydrazine in atazanavir metabolism. *Drug Metab Dispos* **39**:394–401.
- Luo G, Cunningham M, Kim S, Burn T, Lin J, Sinz M, Hamilton G, Rizzo C, Jolley S, and Gilbert D, et al. (2002) CYP3A4 induction by drugs: correlation between a pregnane X receptor reporter gene assay and CYP3A4 expression in human hepatocytes. *Drug Metab Dispos* **30**:795–804.
- Ma X, Chen C, Krausz KW, Idle JR, and Gonzalez FJ (2008) A metabolomic perspective of melatonin metabolism in the mouse. *Endocrinology* **149**:1869–1879.
- Meermann B, Bockx M, Laenen A, Van Looveren C, Cuyckens F, and Vanhaecke F (2012) Speciation analysis of bromine-containing drug metabolites in feces samples from a human in vivo study by means of HPLC/ICP-MS combined with on-line isotope dilution. *Anal Bioanal Chem* **402**:439–448.
- Mesens N, Verbeeck J, and Rouan M (2007) Elucidating the role of M2 in the preclinical safety profile of TMC207, in *38th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease*; 2007 Nov 8–12; Cape Town, South Africa.
- Mukherjee JS, Rich ML, Socci AR, Joseph JK, Virú FA, Shin SS, Furin JJ, Becerra MC, Barry DJ, and Kim JY, et al. (2004) Programmes and principles in treatment of multidrug-resistant tuberculosis. *Lancet* **363**:474–481.
- O'Brien PJ, Siraki AG, and Shangari N (2005) Aldehyde sources, metabolism, molecular toxicity mechanisms, and possible effects on human health. *Crit Rev Toxicol* **35**:609–662.
- Uchida K (2000) Role of reactive aldehyde in cardiovascular diseases. *Free Radic Biol Med* **28**: 1685–1696.
- World Health Organization (2013) *The Use of Bedaquiline in the Treatment of Multidrug-Resistant Tuberculosis: Interim Policy Guidance*, World Health Organization, Geneva, Switzerland.

Address correspondence to: Dr. Xiaochao Ma, Center for Pharmacogenetics, Department of Pharmaceutical Sciences, School of Pharmacy, 3501 Terrace Street, University of Pittsburgh, Pittsburgh, PA 15261. E-mail: mxiaocha@pitt.edu