HHS Public Access

Author manuscript

Int J Tuberc Lung Dis. Author manuscript; available in PMC 2017 November 24.

Published in final edited form as:

Int J Tuberc Lung Dis. 2017 September 01; 21(9): 1069–1070. doi:10.5588/ijtld.17.0408.

An LC-MS/MS-based method to analyze the anti-tuberculosis drug bedaquiline in hair

John Metcalfe[*], **Roy Gerona**[†], **Anita Wen**[†], **Peter Bacchetti**[‡], and **Monica Gandhi**[§]

Roy Gerona: Roy.Gerona@ucsf.edu

[*]Curry International Tuberculosis Center, Division of Pulmonary and Critical Care Medicine, San Francisco General Hospital, University of California, San Francisco, San Francisco, CA, USA

[†]Maternal-Fetal Medicine Division, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, CA, USA

[‡]Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, USA

[§]Division of HIV, Infectious Diseases and Global Medicine, Department of Medicine, University of California, San Francisco, San Francisco, CA, USA

Bedaquiline (BDQ), a diarylquinoline demonstrating significant bactericidal and sterilizing activity, has been approved by the US Food and Drug Administration for the treatment of multidrug-resistant tuberculosis (MDR-TB).[1] QT-interval prolongation and an average terminal elimination half-life of 132 days, leading to potentially long durations of subtherapeutic drug concentration, remain points of concern.[2] Given the importance of drug adherence and exposure monitoring, it is important to develop novel methods of therapeutic drug monitoring, where drug concentrations are measured in a biomatrix. While drug plasma levels represent 'snapshots' of exposure, drug levels in hair reflect uptake from the systemic circulation over weeks to months.[3] Furthermore, hair is collected non-invasively, and can be stored and shipped at ambient temperature without biohazard precautions.

Our group has established expertise in monitoring antiretroviral (ARV) drug exposure[4] and anti-tuberculosis drug exposure in small-volume hair samples, and we have demonstrated strong relationships between hair levels and treatment outcomes in the context of human immunodeficiency virus infection.[5] We describe here the development of a quantitative assay for measuring BDQ in small hair samples using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system (Agilent LC 1260-AB Sciex API 5500, Agilent Technologies, Santa Clara, CA; AB Sciex, Foster City, CA, USA).

Sample preparation consisted of hair pulverization using an Omni Bead Ruptor homogenizer (Omni Inc, Kennesaw, GA, USA), extraction with methanol, evaporation, and reconstitution to 20% (v/v) methanol with 1% formic acid. After injection of the sample extract (10 μl) into the LC-MS/MS, the analytes were separated by gradient elution on a Phenomenex Synergi Polar RP column (2.1 × 100 mm, 2.5 μm particle size; Phenomenex, Torrance, CA, USA)

Conflicts of interest: none declared.

using water with 1% formic acid as mobile phase A (MPA) and acetonitrile with 0.4% formic acid as mobile phase B (MPB). The gradient for analyte separation consisted of 5% MPB at 0–0.3 min, gradient to 100% MPB from 0.3 to 6.8 min, and 5% MPB at 6.81–14 min. Mass spectrometric detection was achieved with electrospray ionization in positive polarity, and mass scanning was performed via multiple reaction monitoring (MRM). BDQ was monitored using two transitions (quantifier 557.1–58.1 m/z; qualifier 557.1–329.9 m/z), and retention time. Quantitation was performed by isotope dilution using deuterium-labeled standard, BDQ d6, monitored using the transition 562.2–64.1 m/z.

Using the LC-MS/MS method described above, we extracted and quantified BDQ from hair specimens in four patients from South Africa on directly observed MDR-TB treatment. Each participant provided written informed consent, and ethical approval was obtained from the University of Cape Town Human Research Ethics Committee (187/2016) and the University of California, San Francisco Human Research Protection Program (CHR#15-17755).

Using a 12-point calibration curve, the assay showed high sensitivity (lower limit of quantification 0.005 ng BDQ/mg hair) and a wide linear dynamic range (0.005–20 ng/mg, average R^2 = 0.996, % coefficient of variation [CV] = 0.37%) using 20 strands of hair of ~2.5 cm in length (~2 mg). The within-run precision (five intra-subject iterations) of our method was within 10% CV (5.25–9.11%). All four patients had BDQ hair levels within the assay's linear dynamic range, at 6.49, 1.35, 5.63, and 2.89 ng/mg for patients 1–4, respectively.

In conclusion, we have developed an assay to accurately and non-invasively determine long-term exposure to BDQ in small hair samples as a metric of adherence and exposure. Full analytic validation of this assay and testing of its utility in clinical settings are underway.

Acknowledgments

Funding for this work was provided by NIH/NIAID R01 AI123024 (PI Metcalfe, Gandhi).

References

1. US Food and Drug Administration. TMC207 (Bedaquiline): treatment of patients with MDR-TB. Silverspring, MD, USA: FDA; 2012. Anti-infective Drugs Advisory Committee Meeting Briefing Document.
2. Dheda K, Esmail A, Limberis J, Maartens G. Selected questions and controversies about bedaquiline: a view from the field. Int J Tuberc Lung Dis. 2016; 20:24–32.
3. Beumer J, Bosman I, Maes R. Hair as a biological specimen for therapeutic drug monitoring. In J Clin Pract. 2001; 55:353–357.
4. Gandhi M, Glidden DV, Mayer K, et al. Association of age, baseline kidney function, and medication exposure with declines in creatinine clearance on pre-exposure prophylaxis: an observational cohort study. Lancet HIV. 2016; 3(11):e521–e528. [PubMed: 27658870]
5. Gandhi M, Ameli N, Bacchetti P, et al. Atazanavir concentration in hair is the strongest predictor of outcomes on antiretroviral therapy. Clin Infect Dis. 2011; 52:1267–1275. [PubMed: 21507924]