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Ethnic and genetic factors in methadone pharmacokinetics: A population pharmacokinetic study☆

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ABSTRACT

Background: Treatment of opiate use disorders with methadone is complicated by wide interindividual variability in pharmacokinetics. To identify potentially contributing covariates in methadone pharmacokinetics, we used population pharmacokinetic modeling to estimate clearance (CL/*F*) and volume of distribution (*V*/*F*) for each methadone enantiomer in an ethnically diverse methadone maintained population.

Methods: Plasma levels of the opiate-active *R*-methadone and opiate-inactive *S*-methadone were measured in 206 methadone maintained subjects approximately two and twenty-three hours after a daily oral dose of rac-methadone. A linear one-compartment population pharmacokinetic model with first-order conditional estimation with interaction (FOCE-I) was used to evaluate methadone CL/*F* and *V*/*F*. The influence of covariates on parameter estimates was evaluated using stepwise covariate modeling. Covariates included ethnicity, gender, weight, BMI, age, methadone dose, and 21 single nucleotide polymorphisms in genes implicated in methadone pharmacokinetics.

Results: In the final model, for each enantiomer, Hmong ethnicity reduced CL/*F* by approximately 30% and the rs2032582 (*ABCB1* 2677G > T/A) GG genotype was associated with a 20% reduction in CL/*F*. The presence of the rs3745274 minor allele (*CYP2B6* 515G > T) reduced CL/*F* by up to 20% for *S*-methadone only. A smaller effect of age was noted on CL/*F* for *R*-methadone.

Conclusion: This is the first report showing the influence of the rs2032582 and rs3745274 variants on methadone pharmacokinetics rather than simply dose requirements or plasma levels. Population pharmacokinetics is a valuable method for identifying the influences on methadone pharmacokinetic variability.

1. Introduction

The misuse of and dependence on opiates is associated with significant morbidity and mortality through overdose and infectious diseases transmitted by injection drug use (Degenhardt et al., 2009). For more than 40 years, the long-acting synthetic opioid methadone has played a central role in the treatment of opiate dependence (Kleber, 2008).

Despite its effectiveness in the treatment of opiate use disorders, methadone is often difficult to use due to its highly variable pharmacokinetics. Estimates of methadone's clearance, volume of distribution, and half-life range from 5.9–13 l/h, 189–470 l, and 15–207 h, respectively (Eap et al., 2002). This difficulty is apparent in the significant rise in methadone associated mortality primarily seen when prescribed for pain by physicians who likely are less familiar with this variability than physicians within highly regulated methadone maintenance settings (Center for Substance Abuse Treatment, 2007). While training in safe prescribing strategies for methadone has resulted in reduced mortality, methadone remains a medication which has highly variable pharmacokinetics making it difficult to devise standard dosing regimens informed by therapeutic drug monitoring (Strang et al., 2010).

Methadone is a racemic mixture whose *R*-enantiomer provides the therapeutic effect at mu-opioid receptors, while both the *R*- and *S*-enantiomers are weak *N*-methyl-D-aspartate (NMDA) receptor antagonists (Eap et al., 2002). Most studies of methadone

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pharmacokinetics have evaluated only total methadone levels; however, it appears that there is also variability in pharmacokinetics within and between enantiomers (*R*-methadone: clearance 4–9.6 l/h, volume of distribution 96–469 l, half-life 24–48 h; *S*-methadone: clearance 7.7–20 l/h, volume of distribution 259–273 l, half-life 20–40 h), which may complicate interpretation of studies assessing the pharmacokinetics of total methadone only.

Population pharmacokinetics (POPPK) is a useful and valid approach toward quantifying drug exposure–clinical response relationships (Food and Drug Administration, 1999; Sheiner and Ludden, 1992). Unlike traditional pharmacokinetic studies, which gather dense data that assess individual variability in drug kinetics, POPPK can use sparse data to model measures of drug exposure and identify factors (e.g., ethnicity, gender, age, weight) that influence variability in drug concentrations across populations (Sheiner et al., 1977).

While the POPPK approach has been validated for methadone maintained individuals there are no studies from large or diverse populations (Foster et al., 2004; Rostami-Hodjegan et al., 1999; Wolff et al., 1997). In fact, over 90% of the subjects in previous POPPK studies of methadone were Caucasian and none of the studies were conducted within a United States population. With larger more diverse sample sizes, variables that contribute to methadone pharmacokinetics (e.g., ethnicity) and treatment outcome may be identified. Based on our previous observations that methadone maintained ethnic Hmong from Laos are on a lower mean dose of methadone yet achieve greater treatment response than do non-Hmong attending the same clinic (Bart et al., 2012), we hypothesized that POPPK could detect decreased methadone clearance in Hmong compared to non-Hmong.

2. Methods

2.1. Subjects

Methadone maintained patients enrolled in a single urban outpatient addiction medicine clinic were recruited into two separate POPPK studies: a cross-sectional study requiring patients to have been on methadone for at least two months without dose change during the previous five days and a prospective study that recruited patients during their first week on methadone and followed them at 1, 3, 6, and 12 months. The prospective study was closed to enrollment after the first 14 subjects due to slow accrual and these subjects were followed as per protocol but once they met inclusion criteria for the cross-sectional study, their prospective data were combined with the cross-sectional subjects in creating the population pharmacokinetic model.

As per Federal criteria, all subjects on methadone maintenance were at least 18 years of age and had met Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for opiate dependence of at least one year duration prior to initiating methadone. Subjects were excluded if they were unable or unwilling to provide informed consent, had decompensated liver disease, were in the second or third trimester of pregnancy, or were taking medications known to alter methadone pharmacokinetics (specifically, phenytoin, rifampin, or highly active antiretroviral therapy for HIV). The study was approved by the Human Subjects Research Committee of the Hennepin County Medical Center and conducted in accordance with the Helsinki Declaration of 1975 (as revised in 1983). Because data included sensitive psychiatric and drug related information a Certificate of Confidentiality was obtained from the National Institutes of Health National Institute on Drug Abuse. The Hmong speaking population was not literate in English or written Hmong, however, consent forms were translated into Hmong and then read to patients by native Hmong speakers in the presence of a study coordinator who could answer questions about the protocol.

Two hundred twenty-four subjects consented to participate in this study. Six subjects did not show up for their study date, two withdrew consent, seven had poor venous access, and three were excluded because their methadone dose had changed within five days of study. Thus, 206 subjects and 441 plasma samples (including prospectively collected specimens from 13 subjects) are included in the analyses.

2.2. Procedures

After signing informed consent, 10 ml of venous blood was drawn into lithium heparin blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) approximately 22–24 h after the previous day's dose of methadone (i.e., roughly trough) and then again 2–4 h after taking their daily methadone dose (i.e., roughly peak). With separate consent, two 10 ml venous blood samples (15% EDTA tubes, Tyco Healthcare Group LP, Mansfield, MA) were collected for DNA isolation and analysis. In a few instances when venous access for one of these time points could not be obtained, a peak or trough sample was taken on a later date. Date, time, and amount of each methadone dose were obtained from a real-time medication dispensing software system (Methasoft, Netalytics, Greer, SC) and dates and times of all blood samples were maintained in a Microsoft Access database. All doses of methadone were consumed under direct supervision on the day of study and more than 95% of the previous day doses were also directly observed (exceptions were for Sunday doses when patients were studied on a Monday).

Subject weight and height were collected on a standard clinical scale (Seca Model 700, Seca Corporation, Hanover, MD) for body mass index (BMI) calculation as weight in kilograms/height in meters^2. Subjects self-identified ethnicity and medications they were taking. Subjects also provided a urine specimen for drug testing and completed the Structured Clinical Interview for DSM-IV (First et al., 1998) to confirm opiate dependence diagnosis and the Symptom Checklist-90 to evaluate for ongoing psychopathology (Derogatis et al., 1973). All interviews were conducted by a single trained master-level research coordinator. For Hmong subjects, interviews were conducted with the assistance of an interpreter knowledgeable in medical and drug use terminology.

2.3. Assays

Blood samples were placed on ice and, within 45 min of being drawn, were centrifuged at 2000 × *g* for 15 min at 4° centigrade for plasma separation. Plasma was immediately stored in 2.0 ml Nunc Cryotubes (Thermo Fisher Scientific, Rochester, NY) at −80° centigrade until analyzed. Plasma levels of each methadone enantiomer were determined using an LC–MS/MS protocol adapted from Liang et al. (Liang et al., 2004).

LC–MS/MS was performed using a TSQ Quantum Classic LC–MS/MS (Thermo Scientific, Waltham, MA) with Agilent 1200 HPLC (Agilent, Santa Clara, CA) and a Chiral-AGP column (5 cm × 2.0 mm, 5 μm particle size, Regis Technologies, Morton Grove, IL). Calibration and quality control using *R*- and *S*- methadone and their D_3-isotope counterparts (Cerilliant, Round Rock, TX) revealed an assay lower level of quantitation of 2.75 ng/ml for *R*-methadone and 2.25 ng/ml for *S*-methadone and a linear range of detection measured between 2.75–687 ng/ml and 2.25–565 ng/ml, respectively. Although not tested beyond these ranges, Liang et al. (2004) found this methodology to be linear up to 1000 ng/ml for each enantiomer. Between and within assay variability percent coefficient of variation were below 6% for both enantiomers.

Urine specimens collected on day of study were analyzed for amphetamine, benzodiazepine, barbiturates, cocaine, and opiates using a commercial immunoassay (EMIT, Beckman, Brea, CA). The presence of methadone metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in urine was also determined (CEIDA, Microgenics, Fremont, CA). All urine drug screening was performed on site in a Clinical Laboratory Improvement Amendments (CLIA) and College of American Pathologists certified laboratory at the Hennepin Faculty Associates (Minneapolis, MN).

2.4. Genetic analysis

Whole blood drawn for genetic analysis was immediately shipped to the Rutgers Cell and DNA Repository for DNA extraction. Purified DNA (mean concentration 116 ng/μl) was shipped to the University of Minnesota for genotyping. Based on published literature, twenty-one single nucleotide variants across a number of genes with known functional effect or that have been explored in previous methadone or opiate use disorder studies were selected for analysis using Sequnom's (San Diego, CA) iPLEX Gold reaction and MassARRAY System for MALDI–TOF (matrix-assisted laser desorption ionization—time of flight) mass spectrometry based genotyping[1].

2.5. Population pharmacokinetic analysis

For determination of population pharmacokinetics, a nonlinear mixed-effects modeling (NONMEM) approach was used with a sparse sampling design including two time points, roughly 2–4 h and 22–24 h after once daily methadone dosing. *R*- and *S*-methadone were analyzed separately. A one-compartment model with first order absorption and elimination (ADVAN2 TRANS2) was used with the NONMEM 7.2 and PDx-Pop 5.0 (both Icon Development Solutions, Ellicott City, MD) software packages installed on a Gateway computer running the Intel 8 fortran compiler, Xpose 4.3.2 (http://www.xpose.sourceforge.net), and R 2.13.0 (The R Foundation for Statistical Computing, Vienna, Austria). While methadone displays a two-compartment pharmacokinetic profile, modeling this is not feasible with only two time points. However, model mis-specification in this instance would affect only estimates of *Ka*, which we fixed, and would not bias estimates of CL/*F* or *V*/*F* (Kowalski and Hutmacher, 2001). A first-order conditional estimation with

[1] See the Supplementary materials for variant identification, further methods, and primer design by accessing the online version of this paper at http://dx.doi.org and by entering doi:.