Effects of *CYP2B6* allelic variants, infection status, liver fibrosis and steatosis on methadone pharmacokinetics

Andrew H. Talal, MD, MPH[1]; Yuxin Ding[2]; Charles Venuto, PharmD[3]; Lindsay Chakan, PhD[1]; Anthony McLeod, BS[4]; Arpan Dharia, MD[1]; Gene D. Morse, PharmD[5]; Lawrence S. Brown, MD, MPH[4]; Marianthi Markatou, PhD[2]; Evan Kharasch, MD, PhD[6]

[1]Division of Gastroenterology, Hepatology and Nutrition, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY; [2]Department of Biostatistics, School of Public Health and Health Professions, University at Buffalo, Buffalo, NY; [3]University of Rochester, Rochester, NY; [4]START Treatment & Recovery Centers, Brooklyn, NY; [5]NYS Center of Excellence in Bioinformatics and Life Sciences, University at Buffalo, Buffalo, NY; [6]Duke University Medical Center, Durham, NC

The authors have nothing to disclose.

Introduction

- Despite the World Health Organization listing methadone as an essential medication and recent increases in its prescription, selecting an effective dose is challenging due to a narrow therapeutic window.
- Subtherapeutic doses can result in withdrawal symptoms potentiating supplementation by illicit heroin use, while supratherapeutic doses can result in overdose and death.
- A variety of factors may affect methadone metabolism, including chronic liver disease, opioid-related infections (e.g., HIV and hepatitis C virus [HCV]) and their treatments, and genetic variation in the hepatic enzyme responsible for methadone metabolism (cytochrome P450 2B6 [CYP2B6]) to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)[1,2].
- A paucity of data in racial and ethnic minority populations, a substantial percentage of those with opioid use disorder (OUD) on methadone, is an additional challenge to effective dose selection.
- The development of effective methadone dosing algorithms, permissive for precision prescribing, remains a critical unmet medical need.
- Our objective was to determine the effects of *CYP2B6* allelic variability and chronic liver disease due to hepatitis C virus (HCV) and HIV/HCV co-infection on methadone metabolism in a population consisting largely of racial and ethnic minorities.
- We hypothesized that *CYP2B6* loss of function (LOF) alleles would result in decreased methadone metabolism.

Methods

- Pre-dose (trough) plasma was collected from 97 evaluable adults on stable once-daily, oral methadone for OUD.
- We measured (R)- and (S)-methadone and (R)- and (S)-EDDP concentrations as well as methadone metabolism ([R]- and [S]-EDDP/methadone concentration ratio).
- Transient elastography assessed hepatic fibrosis and steatosis.
- Genomic DNA was extracted from whole blood and genotyped for *CPY2B6* single nucleotide polymorphisms.
- We utilized a multivariate linear mixed effects model, with nested random effect structure, to analyze the effects of multiple predictors (sex, body mass index [BMI], *CYP2B6* genotype, concomitant medication) on plasma methadone metabolism. Infection status was a random effect nested within recruitment sites.
  - Significance was set at $p<0.05$.
- Outcome variables were (R)-EDDP/methadone concentration and (S)-EDDP/methadone concentration.
  - Data were transformed using natural logarithms.

Results

Demographic and Medical Characteristics of the Study Population

	Variable	Infection status: HCV-mono n=39 (40%)	HCV/HIV n=19 (20%)	Uninfected n=39 (40%)	Total n=97
Demographic Characteristics	**Sex**				
	Male	28 (72%)	11 (58%)	17 (44%)	56 (58%)
	Female	11 (28%)	8 (42%)	22 (56%)	41 (42%)
	Race				
	Black or African American	22 (56%)	9 (47.5%)	28 (72%)	59 (61%)
	Caucasian	13 (33%)	9 (47.5%)	6 (15%)	28 (29%)
	Others	4 (11%)	1 (5%)	5 (13%)	10 (10%)
	Ethnicity				
	Non-Hispanic or Latino	24 (62%)	11 (58%)	31 (79%)	66 (68%)
	Hispanic or Latino	15 (38%)	8 (42%)	8 (21%)	31 (32%)
	Age, years				
	Mean (SD)	56 (9.07)	57 (8.27)	51 (10.51)	54 (9.75)
	Median (IQR)	58 (13)	60 (13)	53 (11)	56 (14)
Medical Characteristics	**Body Mass Index (BMI), kg/m²**				
	Mean (SD)	26.30 (4.94)	23.10 (3.07)	27.96 (6.00)	26.34 (5.37)
	Median (IQR)	25.63 (7.30)	22.86 (2.99)	28.53 (9.12)	25.06 (7.93)
	***CYP2B6* Allele**				
	Normal function	15 (39%)	7 (37%)	12 (31%)	34 (35%)
	Loss of function	22 (56%)	12 (63%)	26 (67%)	60 (62%)
	Gain of function	2 (5%)	0	1 (2%)	3 (3%)
	Concomitant Medication				
	No CYP2B6 inducer or inhibitor	37 (95%)	11 (58%)	39 (100%)	87 (90%)
	CYP2B6 Inducer	2 (5%)	8 (42%)	0	10 (10%)
	Fibrosis Stage*				
	F0-F2	22 (56%)	13 (68%)	33 (87%)	68 (71%)
	>F2	17 (44%)	6 (32%)	5 (13%)	28 (29%)
	Steatosis Level*				
	S0	25 (64%)	14 (74%)	19 (50%)	58 (61%)
	S1	4 (10%)	0	7 (18%)	11 (11%)
	S2-S3	10 (26%)	5 (26%)	12 (32%)	27 (28%)

*One participant has median LSM and CAP value reported as 0, and is excluded from the calculation of descriptive statistics with respect to fibrosis level and steatosis level. Steatosis levels S2 and S3 are combined since there are only 2 participants with S2 stage.

Distribution of Single Nucleotide Polymorphisms and Relationship to *CYP2B6* Genotype among Study Participants

Genotype	rs3745274 genotype (516G>T)	rs2279343 genotype (785A>G)	rs28399499 genotype (983T>C)	rs3211371 genotype (1459C>T)	Count (Frequency)
*1/*1	GG	AA	TT	CC	24 (25%)
*1/*4	GG	GA	TT	CC	1 (1%)
*1/*5	GG	AA	TT	TC	3 (3%)
*1/*6	GT	GA	TT	CC	33 (34%)
*1/*7	GT	GA	TT	TC	7 (7%)
*1/*18	GG	AA	CT	CC	9 (9%)
*4/*6	GT	GG	TT	CC	2 (2%)
*6/*16	GT	GA	CT	CC	6 (6%)
*6/*6	TT	GG	TT	CC	12 (13%)

Distribution of Participants with Respect to Genotype and Genotype by Race, Ethnicity, and Sex

Function	Genotype		Race: Black or African American	Race: Caucasian	Race: Others	Ethnicity: Non-Hispanic or Latino	Ethnicity: Hispanic or Latino	Sex: Male	Sex: Female
Normal Function	*1/*1	24 (25%)	14 (58%)	9 (38%)	1 (4%)	16 (67%)	8 (33%)	13 (54%)	11 (46%)
	*1/*5	3 (3%)	3 (100%)	0 (0%)	0 (0%)	3 (100%)	0 (0%)	2 (67%)	1 (33%)
	*1/*7	7 (7%)	1 (14%)	5 (72%)	1 (14%)	3 (43%)	4 (57%)	1 (14%)	6 (86%)
Gain of Function	*1/*4	1 (1%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)
	*4/*6	2 (2%)	0 (0%)	2 (100%)	0 (0%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)
Loss of Function	*1/*18	9 (9%)	7 (78%)	1 (11%)	1 (11%)	6 (67%)	3 (33%)	8 (89%)	1 (11%)
	*1/*6	33 (34%)	20 (61%)	7 (21%)	6 (18%)	23 (70%)	10 (30%)	21 (64%)	12 (36%)
	*6/*16	6 (6%)	6 (100%)	0 (0%)	0 (0%)	6 (100%)	0 (0%)	2 (33%)	4 (67%)
	*6/*6	12 (13%)	7 (58%)	4 (34%)	1 (8%)	7 (58%)	5 (42%)	8 (67%)	4 (33%)

Methadone Disposition and Metabolism

	Variable	Infection status: HCV-mono n=39 (40%)	HCV/HIV n=19 (20%)	Uninfected n=39 (40%)	Total n=97
Methadone Disposition	**Methadone dose**				
	Mean (SD)	92.56 (50.85)	85.53 (38.22)	95.64 (48.90)	92.42 (47.51)
	Median (IQR)	80 (72.5)	80 (50)	90 (72.5)	80 (60)
	Dose normalized (R)-Methadone concentration				
	Mean (SD)	7.56(5.66)	7.42(4.67)	5.62(2.29)	6.75(4.44)
	Median (IQR)	6.06(4.86)	6.02(1.93)	5.61(3.00)	5.96(2.94)
	Dose normalized (S)-Methadone concentration				
	Mean (SD)	6.91(5.74)	6.86(5.82)	5.96(3.27)	6.51(4.88)
	Median (IQR)	5.61(4.98)	4.87(2.74)	5.51(4.30)	5.41(4.41)
	Dose normalized (R)-EDDP concentration				
	Mean (SD)	0.63(0.59)	0.54(0.40)	0.42(0.18)	0.53(0.43)
	Median (IQR)	0.45(0.39)	0.42(0.23)	0.40(0.16)	0.42(0.25)
	Dose normalized (S)-EDDP concentration				
	Mean (SD)	0.81(0.82)	0.72(0.57)	0.58(0.26)	0.70(0.60)
	Median (IQR)	0.56(0.49)	0.57(0.34)	0.55(0.23)	0.56(0.32)
Methadone Metabolism	**(R)-EDDP/methadone concentration**				
	Mean (SD)	0.09 (0.05)	0.07 (0.03)	0.08 (0.03)	0.08 (0.04)
	Median (IQR)	0.08 (0.04)	0.07 (0.02)	0.08 (0.03)	0.08 (0.03)
	(S)-EDDP/methadone concentration				
	Mean (SD)	0.14 (0.10)	0.11 (0.04)	0.12 (0.05)	0.12 (0.07)
	Median (IQR)	0.12 (0.09)	0.11 (0.04)	0.11 (0.07)	0.11 (0.07)

Estimates of Coefficients for Fixed Effects

$\log_e$([R]-EDDP/methadone concentration)

Effect		Estimate	Standard Error	Pr > |t|	95% Confidence Interval
Intercept		-2.139	0.244	<.0001	(-2.620, -1.657)
Sex	Female	0.216	0.094	0.023	(0.031, 0.400)
	Male	0	.	.	.
BMI		-0.016	0.009	0.068	(-0.033, 0.001)
Genotype	Gain of function	-0.072	0.249	0.772	(-0.564, 0.420)
	Loss of function	-0.168	0.095	0.078	(-0.354, 0.019)
	Normal function	0	.	.	.

$\log_e$([S]-EDDP/methadone concentration)

Effect		Estimate	Standard Error	Pr > |t|	95% Confidence Interval
Intercept		-1.786	0.293	<.0001	(-2.365, -1.207)
Sex	Female	0.223	0.114	0.053	(-0.003, 0.449)
	Male	0	.	.	.
Genotype	Gain of function	-0.08	0.307	0.796	(-0.685, 0.526)
	Loss of function	-0.293	0.116	0.013	(-0.522, -0.063)
	Normal function	0	.	.	.

References

1. Kharasch, E. D., K. J. Regina, J. Blood and C. Friedel (2015). "Methadone Pharmacogenetics: CYP2B6 Polymorphisms Determine Plasma Concentrations, Clearance, and Metabolism." Anesthesiology 123(5): 1142-1153.
2. Kharasch, E. D. and K. Stubbert (2013). "Role of cytochrome P4502B6 in methadone metabolism and clearance." J Clin Pharmacol 53(3): 305-313.

Results Summary

- **Influence of sex**:
  - (R)-methadone metabolism was significantly greater in females compared to males (1.24 greater (R)-EDDP/methadone concentration ratio, p=0.023).
  - Effect on (S)-methadone metabolism provides a non-significant p-value of 0.053.
- **Influence of *CYP2B6* LOF alleles**:
  - (S)-methadone metabolism was significantly lower in participants with LOF versus normal function alleles (1.34 lower (S)-EDDP/methadone concentration ratio, p=0.013).
  - Effect on (R)-methadone metabolism provides a non-significant p-value of 0.078.
- **Concomitant medication by infection status interaction**:
  - Strongly significant p-value of 3.13×10^{-6} indicates presence of subgroup and population heterogeneity.
- The only significant random effects were HCV-monoinfected participants from one clinic (p=0.049).
- Fibrosis and steatosis had no significant effect on methadone metabolism.
- The most common alleles in this largely racial and ethnic minority population were associated with LOF.
- *6/*16 and *1/*18 genotypes were almost exclusively found among study participants of African origin. Other genotypes also appeared to segregate according to Caucasian or African origin.

Conclusions

- Effective methadone dosing requires development of dosing algorithms in order to deliver individualized treatments.
- Inclusion of African-origin populations in genetic studies is essential as many alleles are race-specific.
- We found that (R)-methadone metabolism was significantly affected by female sex. BMI and *CYP2B6* LOF alleles, while significant at 0.1 level, did not reach significance at 0.05.
- (S)-methadone metabolism was significantly affected by *CYP2B6* LOF alleles. Sex had an effect at p-value of 0.1.
- Methadone metabolism appeared to be less in males and individuals with LOF alleles. These parameters should be incorporated into multivariate models, along with other predictors (e.g., BMI), to create dosing algorithms.
- The main limitations of this study include a small sample size and the cross-sectional design, which limited investigation of formal pharmacokinetic relationships.
- Development of methadone dosing algorithms should facilitate medication delivery, improve patient satisfaction, and prevent overdose.

University at Buffalo The State University of New York

Research reported in this work was support from the University of Rochester Center for AIDS Research (4P30AI078498-09), the National Institute of Allergy and Infectious Diseases (K23 AI108355), NIH DA042985, and the Kaleida Health Foundation Troup Fund.