Evaluating the Relationship of Methadone Concentrations and EDDP Formation in Chronic Pain Patients

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Methadone is used to treat moderate to severe pain in patients not responsive to non-narcotic analgesics and for maintenance treatment of opioid addiction. Methadone is primarily metabolized by N-demethylation to an inactive metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidene (EDDP) by CYP3A4 and CYP2B6. Establishing expected concentrations for metabolism of methadone to EDDP using urine excretion data may be useful for monitoring “medications” and toxicity.

Urine specimens from chronic pain patients were collected during routine clinic visits. Methadone and EDDP were quantified by liquid chromatography–tandem mass spectrometry. Approximately 8,000 subjects who reported taking methadone had creatinine concentrations ≥20 mg/dL, and excreted concentrations of methadone and EDDP above ≥100 ng/mL were selected.

The median methadone urine concentration was 3.03 mg/g cr. Ninety-five percent of the population had concentrations between 0.175 and 20.9 mg/g cr. EDDP was, on average, twice the methadone concentration. The wide variance in relationship of methadone to its metabolite was not concentration-dependent. Variability between subjects was larger than variability within subjects. As the urinary pH increased, the proportion of excreted EDDP increased, implying a preferred excretion of EDDP.

Introduction

Methadone is widely used in pain management (1, 2). The principal therapeutic uses of the μ-agonist methadone are for moderate to severe pain not responsive to non-narcotic analgesics and for maintenance treatment of opioid addiction. Unlike the other opioids, methadone is a racemic mixture in which the l-isomer gives methadone its opioid activity and the d-isomer is an N-methyl-D-aspartate (NMDA) receptor antagonist, which makes methadone an effective medication for neuropathic pain (3). This unique mechanism makes it an attractive analgesic for a mixture of nociceptive pain and neuropathic pain. In addition, methadone is an option for patients with true morphine allergy, or renal dysfunction, and its low cost makes it affordable (4). However, methadone’s pharmacokinetic and pharmacodynamic properties are causes for concern about serious and life-threatening side effects. Among the opioids, methadone has been implicated in more unintentional deaths than any other opioid (5). Methadone has a short analgesic half-life of 6–12 hours, but has a variable elimination half-life of 12–150 hours because of its lipid solubility and redistribution into fat (5). Patients may experience delayed heavy sedation and respiratory depression three to five days after initiating methadone therapy or adjusting the dose. Methadone can be an effective pain medication if prescribed and monitored by practitioners who are familiar with its use. Further understanding of metabolism and newer approaches to patient monitoring and toxicity prediction should be explored to provide additional tools for these practitioners. Methadone is primarily metabolized by N-demethylation to an inactive metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidene (EDDP) (6, 7) by CYP3A4, CYP2B6, and with minor activity at CYP2D6 (8–11) and CYP2C19 (12, 13) (Figure 1). Both methadone and EDDP are excreted in urine. Due to methadone’s differential absorption, distribution, metabolism and excretion profile, it is important to assess intra-patient and inter-patient variability in pain patients and to establish a predictive model for monitoring urine methadone and EDDP concentrations.

Urine drug testing is a component of monitoring patients on chronic opioid therapy (5, 14). The availability of a large number of quantitative urine drug test results for methadone and its metabolite EDDP made it possible to study the relationship of methadone to its metabolite in this patient population. The purpose of the first part of this study was to assess the relationship of methadone to its metabolite using urinary excretion data among pain patients. The goal was to determine reference ranges for drug and metabolite concentrations as well as metabolic capacity (measured by the metabolic ratio). The purpose of the second part of the study was to assess the relationship of methadone to EDDP between and within subjects in a population of pain patients. Assessing the amount of variability between and within patients in their relative concentrations of methadone and EDDP may help reduce the possibility of toxicity and explain the variability in efficacy. In essence, establishing predictive methadone excretion models from urine sample data would help physicians verify whether their patients fall within the normal range.

Methods

Subjects and specimens

Urine specimens from chronic pain patients prescribed opioid analgesics among other drugs were collected during routine clinic visits. Specimens were mailed to Millennium Laboratories (San Diego, CA) for drug testing and monitoring. Specimens included in this study were collected over a two-year period (from March 2008 to February 2010). An Excel spreadsheet database was created at Millennium Laboratories to document urinary excretion data. This database included specimen identification numbers, date of urine sample collection, subjects’ date of birth, physicians’ practice code, patients’ current medication list, urine creatinine concentration and urine drug concentrations.
Quantitative analysis of specimens

Samples were tested for several drugs in the same run. An Agilent 1200 series binary pump SL liquid chromatography (LC) system, well plate sampler, and thermostatted column compartment paired with an Agilent triple Quadrupole mass spectrometer (MS) and Agilent MassHunter software were used for analysis of methadone and EDDP (Agilent Technologies, Santa Clara, CA). Chromatographic separation was performed using an acetonitrile–formic acid–water gradient running at 0.4 mL/min and a 2.1 × 50 mm, 1.8 µm Zorbax SB C18 column. Mobile phase A was 0.1 percent formic acid in water and B was 0.1 percent formic acid in acetonitrile. The gradient was 15% B at 0.0 min, 50% B at 2.0 min, 100% B at 5.0 min and 100% B at 5.1 min; the stop time was 7.0 min. The column temperature was set to 50°C. Samples were prepared for injection by incubating 25 µL of urine with 50 units of glucuronidase β-glucuronidase Type L-II from Patella vulgata (keyhole limpet) (Sigma-Aldrich Corp., St. Louis, MO) in 50 µL of 0.4M acetate buffer (pH 4.5) for 3 h at 45°C. Five microliters of the solution was injected for each sample.

All spectra were collected using positive electrospray ionization. The optimized instrumental parameters were as follows: gas temperature, 350°C; drying gas, 12 L/min; nebulizer gas (nitrogen), 35 psi (~24,100 Pa); capillary voltage, 3,000 V; and fragmentor voltage, 60 V. Multiple reaction monitoring (MRM) mode was used for quantitation and product ion scan (PIS) mode was used to acquire full scan MS-MS spectra. In PIS mode, MS-MS spectra were collected with collision energy set to 5, 10, 20 and 30 V. Product ion spectra were collected in the range of 50 to 350 m/z. Scan time was set to 500 ms.

In MRM mode, two transitions were used to identify and quantify a single compound. A quantitative transition was used to calculate concentration based on the qualifier ion and a qualitative transition was used to ensure accurate identification of the target compound based on the ratio of the qualifier ion to quantifier ion. The following quantitative transitions were used: methadone-D3 313.2 → 268.2; methadone 310.2 → 265.1; methadone 310.2 → 140; EDDP-D3 281.2 → 237.1; EDDP 278.2 → 234.1, EDDP 278.2 → 186. Dwell times were set to 50 ms.

HPLC-grade water, acetonitrile, methanol and formic acid were obtained from VWR (Westchester, PA). Methadone and EDDP were obtained from Cerilliant Corp (Round Rock, TX). The deuterated internal standards were diluted to 1,000 ng/mL by adding them to synthetic urine (Microgenics Corp., Fremont, CA).

Quantitative analysis was performed using Agilent MassHunter Quantitative Analysis software. A four-point calibration curve was created by using a linear fit and forcing the origin. The calibrators were set at 100, 200, 6,400 and 12,800 ng/mL. The quality control samples were set at 100 and 1,000 ng/mL. Accepted accuracy for calibrators was ± 20 percent of the target value and the coefficient of determination (R²) was required to be greater or equal to 0.99 as verification of linearity and goodness-of-fit. The lower limit of quantitation (LLOQ) for the methadone and EDDP assays were 100 ng/mL. The measured upper limit of linearity for the methadone and EDDP assays were 100,000 ng/mL.

Subject inclusion criteria

Part 1

The following inclusion criteria were used to determine the study populations for Part 1. Sorting methodology is summarized in Figure 2.

(i) Urine creatinine concentrations greater than or equal to 20 mg/mL. This criterion removes dilute urine samples and is consistent with workplace drugs of abuse urine testing guidelines for validating random urine specimens (15).
(ii) Report of methadone (generic and brand names) on the medication list.
(iii) Urine specimens with methadone and EDDP concentrations greater or equal to the LLOQ of 100 ng/mL; i.e., both ≥ 100 ng/mL.
(iv) Subjects with one visit or the first of several visits were defined as the inter-subject population.

Part 2

The following inclusion criteria were used to determine the inter-subject and intra-subject study populations for Part 2.

(i) Urine creatinine concentrations greater than or equal to 20 mg/mL.
(ii) Report of methadone (generic and brand names) on the medication list.
(iii) Urine specimens with methadone and EDDP concentrations greater than or equal to the LLOQ.
(iv) Subjects with one visit or the first of several visits were defined as the inter-subject population.
(v) Subjects with five or more visits were defined as the intra-subject population.

Study component analysis

The metabolic ratio (MR) was defined as the concentration ratio of EDDP to methadone in urine specimens. The MR is unitless and is assumed to represent the subjects’ relationship of methadone to its metabolite at the time of collection.
Methadone exposure was defined as an estimate of the total methadone load and was calculated by adding urinary methadone concentration to its molar equivalent of EDDP. Methadone and EDDP concentrations were corrected (normalized) to grams of excreted creatinine. This technique has been shown to help avoid inappropriate results caused by dilute urine specimens (15–19). Analyte/creatinine ratios were calculated by dividing the analyte level (ng/mL) by the urine creatinine level (mg/dL). The final concentrations were reported as milligram analyte per gram creatinine (mg/g cr).

Ethics
All study data were de-identified before analyses. Institutional Review Board-exempt status was granted by the University of California, San Diego Human Research Protections Program (HRRP).

Statistical methods
Database management, descriptive statistics and graphical analyses were conducted using OriginPro 8.1 (OriginLab Corp., Northampton, MA) and Microsoft Excel 2007 (Microsoft Corp., Redmond, WA) software. To present descriptive statistics and to evaluate normal distribution of analyte concentrations, MR and methadone exposure between and within subjects, the raw data were log-transformed. Log-transformed data were used to perform the calculation of the mean, standard deviation (SD), 95% confidence interval (CI) of the mean, median and interquartile range (IQR). Log-transformed data were expressed as mean ± SD. The 2.5- and 97.5-percentiles were also reported to assess the range in 95% of the population. The log-transformed data were then back-transformed and expressed as the geometric mean ± geometric standard deviation (GSD), geometric median and geometric IQR.

Results: Part 1
Eight thousand, nine hundred subjects reported methadone on the medication list and had creatinine concentrations ≥ 20 mg/dL. Of these, 7,962 subjects with single or first visit were used for analysis. Nine hundred thirty eight subjects with methadone and EDDP below LLOQ were removed.

Range of values
The excretion observations indicated that the range of values was quite large. For that reason, logarithmic representations of the data were used. Figures 3A and 3B are histograms showing the distribution of the urinary concentration of methadone (Figure 3A) and creatinine corrected methadone concentration (mg/g cr) (Figure 3B). The data are plotted logarithmically and the creatinine corrected plot approximates a Gaussian distribution. The geometric mean was 2.66 ± 3.43 mg/g cr and the 95% CI of the mean was 2.59–2.74 mg/g cr. The median was 3.03 mg/g cr, with a 25th percentile of 1.24 mg/g cr and a 75th percentile of 6.25 mg/g cr. The arrows show that 95% of the population has a concentration within the range of 0.175 to 20.9 mg/g cr. These descriptive statistics of urinary methadone concentrations are summarized in Table I.

Figures 3C and 3D are similar histograms showing distribution of the concentration of EDDP (Figure 3C) and EDDP concentration creatinine corrected (mg/g cr) (Figure 3D). The logarithmic plot for the creatinine corrected EDDP approximates a Gaussian distribution. The geometric mean was 4.68 mg/g cr ± 3.06 and the 95% CI of the mean was 4.57–4.80 mg/g cr. The median was 5.28 mg/g cr, with a 25th percentile of 2.47 mg/g cr and a 75th percentile of 10.2 mg/g cr. The arrows show that 95% of the population has an EDDP creatinine corrected concentration within the range 0.36 to 30.2 mg/g cr. These statistics are also summarized in Table I. These descriptive statistics show that the expected range of EDDP excretion is higher than the unchanged methadone by approximately two-fold.

Relationship of methadone to its metabolite EDDP
For this analysis, a plot of log methadone creatinine corrected (mg/g cr) versus log EDDP creatinine corrected (mg/g cr) was...
Figure 3. Distribution of log-transformed methadone concentrations that are above the LLOQ: expected range of methadone concentration values in urine samples in patients taking methadone. (A). Distribution of log-transformed methadone creatinine corrected concentrations that are above the LLOQ; the range within the arrows represents 95% of the study population (B). Distribution of log-transformed EDDP concentrations that are above the LLOQ: expected range of EDDP values in urine samples in patients taking methadone (C). Distribution of log-transformed creatinine corrected EDDP concentrations that are above the LLOQ: expected range of EDDP values in urine samples in patients taking methadone; the range within the arrows represents 95% of the study population (D).

Table 1
Descriptive Statistics of Log Creatinine-Corrected Methadone and EDDP Concentrations Above the LLOQ

<table>
<thead>
<tr>
<th>Descriptive statistics</th>
<th>Methadone geometric data</th>
<th>EDDP geometric data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2.66</td>
<td>4.68</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>3.43</td>
<td>3.06</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.03</td>
<td>1.02</td>
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<tr>
<td>Lower 95% CI of mean</td>
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</tr>
<tr>
<td>Upper 95% CI of mean</td>
<td>2.74</td>
<td>4.80</td>
</tr>
<tr>
<td>Median</td>
<td>3.03</td>
<td>5.28</td>
</tr>
<tr>
<td>2.5-percentile</td>
<td>0.175</td>
<td>0.360</td>
</tr>
<tr>
<td>25-percentile</td>
<td>1.24</td>
<td>2.47</td>
</tr>
<tr>
<td>75-percentile</td>
<td>6.25</td>
<td>10.2</td>
</tr>
<tr>
<td>97.5-percentile</td>
<td>20.9</td>
<td>30.2</td>
</tr>
</tbody>
</table>

The scatter plot shows a positive correlation between log methadone concentration and log EDDP concentration in urine samples ($R^2 = 0.54, \text{slope} = 0.67$). Although metabolite (EDDP) formation appears to be drug (methadone) concentration-dependent, the relationship is not directly proportional (1:1). As shown by the scatter, the variance in the formation of metabolite in the population was large. To further examine the relationship between methadone and EDDP, MR was assessed (Figure 4B). MR represents metabolite–drug ratio [EDDP]/[methadone] and describes subjects’ metabolic capacity. When using MR, each subject becomes its own control. MR corrects for some of the between-patient variance in the previous analysis. The scatter plot shows a negative correlation
between log MR and log methadone concentrations in urine samples \((R^2 = 0.23, \text{ slope} = -0.33)\). That is, metabolite (EDDP) formation appears to be dependent on the drug (methadone) concentration, but the metabolite formation does not increase as quickly as the parent drug.

Concentration dependence of the relationship of methadone to its metabolite EDDP

One of the limits of this study is that the dosage of drug given to the patient is unknown. Therefore, the variance in methadone and EDDP concentrations could be due to different doses. As an attempt to correct for this limitation, the variance in relationship of methadone to its metabolite EDDP was calculated from a narrow range of excreted methadone. For example, a small slice of a range of values around the median methadone concentration from Figure 4A was taken and evaluated. The median log methadone concentration in Figure 4A was 0.481. The slice of \(\pm 0.1 \text{ mg/g cr} \) was taken around that median and consisted of 1,195 urine specimens (15% of the total population). The range of methadone concentrations at the median \(\pm 0.1 \text{ mg/g cr} \) was 0.381 to 0.581 mg/g cr. Furthermore, log EDDP concentrations were evaluated within the slice and ranged from \(-0.713 \) to 1.75 (Figure 4C). The calculated variance in log EDDP concentration at the slice around the median log methadone concentration was 15-fold and followed a Gaussian distribution (Figure 4C), demonstrating that a wide range of metabolite

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Figure 4. Scatter plot of methadone concentration versus EDDP formation: the plot shows a positive correlation, but not a 1:1 proportionality \((R^2 = 0.54, \text{ slope} = 0.67)\) (A). Scatter plot of EDDP–methadone MR as a function of parent drug (methadone) concentration (B). Variability of EDDP around the geometric median of methadone: this histogram represents a Gaussian distribution of metabolite EDDP concentrations from the slice depicted in Figure 4A (C).
(EDDP) is formed even at a set concentration of parent drug (methadone). The study is further limited because the secondary metabolite, EMDP, has not been measured and for plots, such as those in Figures 4A, 4B and 4C, lacking the amount of this metabolite could have a major impact, along with differences in urinary excretion and reuptake and effect of pH.

**Relationship of methadone to its metabolite EDDP as a function of methadone exposure**

Because EDDP is usually excreted at a concentration greater than the methadone concentration, an analysis of the relationship of methadone to its metabolite EDDP was done using the total excreted concentrations of methadone and EDDP combined, termed methadone exposure. Methadone exposure can be estimated in the absence of dosage information by adding urinary methadone and EDDP molar concentrations. Methadone exposure in the inter-subject population ranged from 0.0832 to 229 mg/g creatinine (antilog $-1.08$ to $2.36$) with median methadone exposure of 9.40 mg/g creatinine (antilog $0.973$) (Figure 5A). Using the metabolic ratio as an indicator of the relationship of methadone to its metabolite EDDP and total methadone exposure as an indicator of concentration, the graphic relationship as shown in Figure 5B is flat. That is, no relationship is visible between the two parameters.

![Figure 5.](image-url)
A wide scatter of points is observed around the median value of MR. To calculate the variability in MR with respect to total methadone exposure in typical subjects, a +0.1 slice of log data from Figure 5B was taken around the median methadone exposure and illustrated in a histogram (Figure 5C). This slice consisted of 1,481 urine specimens. The median log MR in Figure 5C was 0.481. The variance in MR observed at the slice around the median total methadone exposure was 33-fold and followed a slightly skewed (mean = 0.26; median = 0.22) Gaussian distribution, which suggests that a wide range of metabolic ratios can be observed at a set concentration of total methadone exposure.

**Excretion as a function of urinary pH**

Previous studies have shown that methadone excretion is pH-dependent. Figure 6 shows the correlation of metabolic ratio ([EDDP]/[Methadone]) with pH. As the pH increases, the proportion of EDDP increases, which implies a preferred excretion of EDDP with increasing pH.

**Results: Part 2**

To assess inter-subject variability, 8,083 subjects who reported taking methadone, had creatinine concentration ≥20 mg/dL and had concentrations of methadone and EDDP above the quantitative limit (≥100 ng/mL) at single or first visits were selected. To assess intra-subject variability, in addition to the preceding criteria, 190 of the 8,083 subjects with ≥5 visits were selected.

**Methadone exposure**

Figure 7A shows the distribution of total drug excreted (defined as methadone exposure) for intra-subject population. Combined methadone exposure was calculated by summation of [EDDP] and [methadone], corrected for [creatinine], and log transformed. The methadone exposure in the intra-subject population ranged from 1.21 to 96.6 mg/g creatinine (antilog 0.08 to 1.98). As expected, the intra-subject population range falls within that of the inter-subject population range.

**Inter-subject and intra-subject variability of the MR**

Similar to methadone exposure, in terms of MR distributions, the individuals representing intra-subject variability (Figure 7B) in their relationship of methadone to its metabolite EDDP are representative of individuals from the overall population. Figure 7B represents the intra-subject MR distribution. In 95% of the population, the MR ranged from 0.61 to 6.73 (antilog –0.22 to 0.83) with a geometric mean of 1.68 ± 1.70. In 95% of the intra-subject population, the MR varies up to 11-fold. Table II provides a summary analysis of descriptive statistics pertaining to inter-subject and intra-subject methadone and EDDP concentrations, MR and methadone exposure.

**Variability in relationship of methadone to its metabolite EDDP**

A single value of log [M], log [E], log MR and log M exposure for each subject was obtained by averaging the log-transformed data from each visit. Back-transformation of logged data was used to obtain geometric values. For the intra-subject population, most of the patients had a narrow standard deviation; however, a significant number of subjects had a wide MR variation. The mean GSD of MR within subjects is 1.73. As a point of reference to represent the overall population, the GSD of MR of the inter-subject population was included; that is, 2.38. Therefore, the variability in methadone relationship of methadone to its metabolite EDDP is 1.38 (2.38/1.73) times greater between subjects than within subjects (Table II).

**Discussion**

Many previous studies have been published on the urinary excretion of methadone and its metabolite EDDP (20, 13, 21, 6, 22, 23). One in particular claimed that plasma concentrations could be calculated from urine concentration data (24). This study provides information on the expected methadone and EDDP concentrations in urine from chronic pain patients taking methadone. Distributions of creatinine-corrected, log-transformed methadone and EDDP concentrations in urine samples that are above the LLOQ are presented in Figures 3A and 3B. Methadone and EDDP concentrations are summarized in Table I. Clinicians can use the geometric data reference from Table I to assess their patients’ urinary excretion profile with respect to a population of 7,962 subjects taking methadone. Patients falling outside the IQR or the 95% of this study’s population should be considered for closer monitoring, because they might be at risk of being undertreated, reaching toxicity or in the more unlikely scenario diverting the drug.
Relationship between methadone and EDDP concentrations

Our results demonstrate a positive relationship between methadone and EDDP concentrations in urine. This is in agreement with the fact that methadone is metabolized to its primary metabolite EDDP via N-demethylation. However, this study also shows that as the amount of methadone increases, a less than one-to-one increase in EDDP is observed, as described by the slope value of $+0.67$ (Figure 4A). The nonproportional changes in metabolite formation might imply saturation. However, saturable metabolic pathway is only one of several potential explanations for a non-proportional relationship between drug and metabolite concentration in the urine samples. Such findings could also suggest saturable methadone reabsorption at higher methadone concentrations. Additionally, we also observed a large variability in the formation of metabolite (EDDP) as a function of a given methadone concentration. The calculated variance in log EDDP concentration at the slice around the median log methadone concentration was 15-fold and followed a Gaussian distribution (Figure 4C). This suggests that a wide range of metabolite (EDDP) is formed even at a set concentration of parent drug (methadone). This is most likely due to the pharmacogenomic differences in the population.

Relationship between methadone and MR

The scatter plot (Figure 4B) of methadone versus metabolic ratio (methadone/EDDP) once again shows that there is not a one-to-one proportionality (slope = $-0.33$). The metabolite (EDDP) formation appears to be greater at lower methadone concentrations and less at higher methadone concentrations.
Relationship between methadone exposure and MR

We estimated urinary methadone exposure in the absence of dosage information by adding urinary methadone and EDDP molar concentrations. Methadone exposure in the inter-subject population ranged from 0.0832 to 229 mg/g creatinine (antilog $\approx 20$-fold), re-oundary variability in plasma concentrations, then one would expect the MR at any given methadone exposure (Figure 5C). We found 33-fold variability in MR around the median methadone exposure concentration. This study concludes that the possible range of methadone metabolite EDDP is inactive (6, 7); therefore, subjects with truly decreased metabolism would show increased pharmacodynamic effects and possible toxicity.

Estimation of ultra-rapid metabolizers

In the same way that genetic distributions of the CYP 3A4, 2B6 and 2D6 genes lead to poor and non-metabolizers, another population metabolizes methadone more quickly than average. This could result in measurements that have detectable EDDP but not methadone. Samples that have lower concentrations of drug may simply be toward the beginning or end of their dose interval and therefore have levels below detection by chance. Meanwhile, one of many explanations for an increase in the number of measurements with detectable methadone but no EDDP at high concentrations of methadone could be poor metabolism or subjects attempting to falsely test positive for methadone by adding the drug to their urine. This would result in high methadone with no metabolite. Importantly, methadone metabolite EDDP is inactive (6, 7); therefore, subjects with truly decreased metabolism would show increased pharmacodynamic effects and possible toxicity.

Inter-subject and intra-subject variability

As a means of representing the relationship of methadone to its metabolite EDDP, this study characterized and compared the MRs from urine samples in inter-subject and intra-subject
populations (Table II). Based on standard deviation values depicted in Figure 7B, this study concludes that the inter-subject variability in the relationship of methadone to its metabolite EDPD is greater than the intra-subject variability. In addition, we found that the relationship of methadone to its metabolite exhibits up to 11-fold variability within subjects. Methadone exposure can be estimated in the absence of dosage information by adding urinary methadone concentration to its molar equivalent of EDPD.

The reason for the large standard deviations of MR compared to the mean estimates could be attributed to the following concepts in the relationship of methadone to its metabolite EDPD: genetic variability in P-glycoprotein activity (up to 11-fold) (27, 28); polymorphisms in either liver or intestinal CYP enzymes (up to 17-fold) (28, 29); variability of urinary excretion of methadone and EDPD (17–57% of given dose) depending on urinary pH (29); concurrent administration of interacting drugs (inducers and inhibitors of liver enzymes) (30–32); and variable "consistency with prescribed medications."

**Conclusion**

Concentrations of EDPD and methadone found in the urine exhibit a large variability in the MR (EDPD/methadone). The amount of EDPD found in the urine and the MR were found to have less than proportional relationships with the amount of methadone in the urine, indicating saturation in the relationship of methadone to its metabolite EDPD or reabsorption pathways of methadone. It is possible to identify subjects found within the sub-population who exhibit unusual metabolite formation as compared to the linear model presented in this study. Variation in analyte concentrations between and within chronic pain patients prescribed methadone were determined, with higher variability observed between subjects. The results presented here may help physicians assess the effectiveness of therapy, assist in establishing a reference range for monitoring urine methadone and EDPD concentrations, and may help to explain methadone-associated adverse events, potential abuse or diversion.

**References**


