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Methadone and Metabolites in Hair of Methadone-Assisted Pregnant Women and Their Infants

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Abstract

Methadone is the recommended pharmacotherapy for opioid-dependent pregnant women. The primary aims of this study were to determine whether a dose-concentration relationship exists between cumulative maternal methadone dose, methadone and metabolite concentrations in maternal hair during pregnancy and whether maternal hair methadone and metabolite concentrations predict neonatal outcomes.

Materials and Methods—Hair specimens were collected monthly from opioid-dependent mothers enrolled in methadone treatment and 4 of their infants. Hair specimens were segmented (3cm), washed (maternal hair only) and analyzed for methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrrolidine (EMDP) by liquid chromatography tandem mass spectrometry.

Results—There was large inter-subject variability and no dose-concentration relationship for cumulative methadone dose and methadone, EDDP, EMDP or total concentrations in hair. For individual women, a positive trend was noted for cumulative methadone dose and methadone and EDDP concentrations in hair. There was a positive linear trend for cumulative methadone dose and EDDP/methadone ratio in maternal hair, perhaps reflecting methadone's induction of its own metabolism. Maternal methadone concentrations were higher than those in infant hair, and infant EDDP hair concentrations were higher than those in maternal hair. Maternal methadone dose, and methadone and EDDP hair concentrations were not correlated with peak infant neonatal

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abstinence syndrome (NAS) scores, days to peak NAS, duration of NAS, time to NAS onset, birth length, head circumference or amount of neonatal morphine pharmacotherapy. Maternal cumulative 3rd trimester methadone dose was positively correlated with infant birth weight.

Conclusion—Methadone and EDDP in pregnant women's hair are markers of methadone exposure and do not predict total methadone dose, nor neonatal outcomes from *in utero* methadone exposure.

Keywords

Methadone; EDDP; Hair; *In utero* exposure; Neonatal outcomes

INTRODUCTION

Methadone-assisted therapy is the standard of care for pregnant opioid-dependent women in the United States.¹ Untreated opioid dependence during pregnancy is accompanied by a risk of adverse maternal and neonatal outcomes including spontaneous abortion, preeclampsia, placental abruption, pre-term delivery, low birth weight, small head circumference, and meconium stained amniotic fluid.^{2–4} Outcomes for opioid-dependent mothers and their prenatally opioid-exposed children are improved with adequate daily methadone dosing, as compared to highly fluctuating opioid concentrations during heroin or prescription drug abuse. Methadone therapy also is associated with negative maternal and neonatal effects, most notably neonatal abstinence syndrome (NAS) in 60–87% of methadone-exposed infants.⁵ NAS is characterized by central nervous system hyperirritability and dysfunction of the autonomic nervous system, gastrointestinal tract, and respiratory system.⁶ When left untreated, NAS can result in difficulty feeding, diarrhea, seizures, weight loss, and/or death.⁶

Hair testing offers advantages over more traditional drug monitoring matrices such as plasma or urine. Hair has a long window of detection (months-years) and collection is non-invasive. Although it is possible to adulterate drug concentrations in hair by bleaching, straightening or other cosmetic procedures, and hair can be cut or shaved, adulteration is apparent in hair, unlike urine adulteration. Dose-concentration relationships of methadone and EDDP in hair of adult patients enrolled in methadone-assisted therapy are reported in the literature, but findings are inconsistent. Some reports document no methadone dose-concentration relationship,^{9–11} while others observed significant correlations.^{12–15} There is one previous investigation of methadone in hair from pregnant women in treatment;¹⁶ however, maternal methadone doses were unknown, precluding investigation of dose-concentration relationships for methadone markers in maternal hair.¹⁶ Another limitation of this earlier study was that insufficient infant hair was available for analysis.¹⁶ Non-invasive hair analysis in pregnant methadone-assisted women offers a unique opportunity to assess dose-concentration relationships in this vulnerable population.

Hair result interpretation is complicated by variable hair growth rates,^{17–21} time delay for drug incorporated in the hair root to reach the scalp for sampling,^{22–23} and hair left on the scalp when sampling.¹⁸ The traditionally accepted hair growth rate is 1.0 cm/month; however, an alternative rate of 1.4 cm/month,^{20–21} is suggested to account for the wide variability in hair growth rates seen in the general population and the lengthened duration of hair's anagen growth phase during pregnancy.²⁴ Additionally, recent reports suggest that on average 0.8cm hair is left on the scalp after sampling with scissors.¹⁸ Assuming a 1.0 cm/month growth rate, this 0.8cm left on the scalp corresponds to 24 days, while employing a 1.4 cm/month growth rate, this same length is equivalent to 18 days. The influence of these factors on methadone dose-hair concentration relationships has not been evaluated.

The primary objectives of this study were to 1) determine whether maternal methadone dose predicts methadone and metabolite concentrations in maternal hair, 2) assess whether methadone markers in maternal hair predict neonatal outcome measures, and 3) evaluate factors of hair growth, time for drug incorporated in the hair root to reach the scalp, and hair left on the scalp on dose-concentration relationships. We hypothesized that the cumulative maternal methadone dose would predict the concentration of methadone and metabolites in maternal and infant hair, and that maternal hair concentrations would predict adverse neonatal outcomes. If this were true, hair testing could improve clinical care of this vulnerable population, and identify children and families requiring additional resources. This investigation is unique as adequate maternal methadone dose, serial quantitative methadone and metabolite hair concentration, and neonatal outcome data are rarely available to test these hypotheses.

MATERIALS AND METHODS

Participants

Opioid-dependent pregnant women 18–40 yrs old enrolled in methadone-assisted pharmacotherapy at the Center for Addiction and Pregnancy (CAP), John Hopkins Bayview Medical Center (JHBMC), Baltimore, Maryland, and provided written informed consent to participate. Women were required to meet the Diagnostic and Statistical Manual of Mental Disorders-IV criteria for current opioid dependence and were enrolled during gestational weeks 8–30. All women received monetary incentive vouchers for urine specimens negative for illicit opiates and cocaine.²⁵ Methadone dosing was flexible (i.e. individualized) and clinically driven by participant response. Institutional Review Boards of JHBMC and the National Institute on Drug Abuse (NIDA) approved the studies.

NAS Assessment and Neonatal Outcome Measures

Infants were hospitalized for at least 4 days after birth per hospital standards. NAS symptoms were evaluated on a 19-item modified Finnegan scale 6–8 times a day.⁵ Oral morphine sulfate drops (0.02 mg morphine/drop) were administered to infants every 3–5 h when 3 consecutive NAS scores were ≥ 8 or 2 consecutive scores were ≥ 12 . Time to NAS onset was defined as days from birth until the first score ≥ 4 , NAS duration was days from first score ≥ 4 until all scores were < 5 , and time to peak NAS score was days from birth to peak score. A score of 4 was selected as a cutoff based on clinical experience.²⁶ Birth weight (g), length (cm) and head circumference (cm) were extracted from hospital records.

Sample Collection and Preparation

Hair samples were collected at enrollment and approximately once each month throughout pregnancy. As each daily methadone dose and time of hair collection were recorded, estimation of the cumulative methadone dose corresponding to each hair sample was possible. Infant hair was collected within three days of delivery. Hair samples were collected with scissors from the posterior vertex, and cut as close to the scalp as possible. Hair specimens were wrapped in aluminum foil with orientation noted.

Certified ($\pm$)-methadone, ($\pm$)-methadone-d₃, ($\pm$)-2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) perchlorate, ($\pm$)-EDDP-d₃ perchlorate standards were purchased from Cerilliant (Round Rock, TX). ($\pm$)-2-ethyl-5-methyl-3,3-diphenylpyrrolidine (EMDP) HCl and ($\pm$)-EMDP-d₃ HCl were obtained from NIDA Research Drug Supply, Research Triangle Institute (Research Triangle, NC).

Maternal hair specimens were segmented (3cm) starting at the root-end and minced finely with scissors. Calibrators and quality control samples (QCs) were prepared in drug-negative

hair (10mg). Ten calibrators were fortified ranging from 0.1 to 50 ng/mg for methadone, EDDP and EMDP. QCs were fortified at 1.0, 10 and 37.5 ng/mg. Calibrators, QCs and maternal hair specimens (10mg) were washed twice with methylene chloride²⁷ and spiked with internal standard (25 μ L 2.0 ng/ μ L methadone-d3, EDDP-d3 and EMDP-d3). Base digestion overnight followed by liquid-liquid extraction was performed according to Wilkins et al.²⁸ Residues were reconstituted with 100 μ L 0.1% aqueous formic acid: acetonitrile (85:15 v/v). Infant hair (10mg) was analyzed without segmentation or washing. Unwashed calibrators, QCs, and additional maternal and infant hair specimens were processed in the same manner without washing.

LC-MS/MS Analysis

A gas chromatography mass spectrometry method by Wilkins et al.²⁸ was adapted for LC-MS/MS with the following modifications. Liquid chromatography was performed with an Agilent 1100 LC system. A YMC ODS-AQ 150 $\times$ 2.0 mm, 3 μ m reverse phase column (Waters, Milford, MA) was employed with gradient elution with 0.1% aqueous formic acid and acetonitrile. Acetonitrile content was held at 30% for 0.3min, increased to 45% at 0.5min, 80% at 5.0min, and 95% at 5.1min, held at 95% for 1.1min, and followed by column equilibration at 30% for 2.8min. Total run time including reconditioning was 10 min. Samples were introduced into a Thermo-Finnigan TSQ 7000 triple quadrupole mass spectrometer with electrospray ionization operating in selected reaction monitoring (SRM) mode. Ion transitions for each analyte were: 310.2 $\rightarrow$ 265.1 for methadone, 313.2 $\rightarrow$ 268.1 for methadone-d3, 278.1 $\rightarrow$ 234.0 for EDDP, 281.1 $\rightarrow$ 234.0 for EDDP-d3, 264.1 $\rightarrow$ 220.0 for EMDP and 267.1 $\rightarrow$ 220.0 for EMDP-d3. Drug concentrations were determined from peak area ratios of analyte to internal standard compared to calibrator curves of peak area ratios to concentrations. Analyte concentrations were linear from 0.1 to 50 ng/g, determined from regression with $1/x^2$ weighting. Calibration curves were highly correlated ($r = 0.997$). Analytical bias was <10% for calibrators and <14% for QCs. Intra-assay (N=6) and inter-assay (N=20) imprecision variability was tested at the three QC concentrations and percent coefficient of variations (%CVs) ranged from 1.0–3.6%, and 3.1–11.6% for all analytes. Recovery of methadone, EDDP and EMDP from hair was similar to Wilkins et al.²⁸ Stability of hair extracts was assessed at the three QC levels (each N=6) after 48 hours at -20°C . Extracted QC concentrations were analyzed immediately after extraction, stored at -20°C for 48 hours and re-analyzed. All QC concentrations were within 10% of the target concentrations.

Data Analysis

Statistics were performed with GraphPad Prism V5.02 from GraphPad Software, Inc, USA. Drug concentrations below the limit of quantification (LOQ) or above the upper limit of quantification (ULOQ) were excluded from statistical analyses.

Methadone and metabolite hair concentrations represent cumulative drug exposure during the hair growth period. Cumulative methadone dose for each proximal segment was calculated from daily methadone administration records. To determine the dosing period represented by the hair segment, several factors were assessed, including variable hair growth rates, time required for drug incorporated from the blood into the hair root to reach the scalp, and hair left near the scalp when sampling. As Figure 1 demonstrates, eight unique dosing periods were evaluated for each proximal segment based on these factors.

Dose-concentration relationships were evaluated by linear regression. For dose-concentration assessments in maternal hair proximal segments, the family-wise alpha level was set to 0.00625 using Bonferroni's principle to account for multiple comparisons. Normality was assessed with visual inspection of box plots and Kolmogorov-Smirnov tests.

Grubbs' test and visual inspection of box plots identified outliers. Matched paired t-tests assessed differences between proximal and distal concentrations and washed and unwashed concentrations. Pearson and Spearman correlations evaluated associations between normally and non-normally distributed variables, respectively. Statistical probability (P) <0.05 was considered statistically significant for these comparisons.

RESULTS

Participants

Twenty-nine women participated in the study. The majority (75.9%) were African American. Women received methadone throughout gestation for a mean ($\pm$ SD) of 18.6 ± 6.5 weeks (range 8–35). Mean daily methadone dose at enrollment was 37.0 ± 15.0 mg (20–90) and at delivery 73.4 ± 18.4 mg (30–110). Generally, daily methadone doses gradually increased over the course of pregnancy; some women received the same daily dose for several months. During the 3rd trimester, women tended to receive their highest daily doses; however, there was large variability. Mean doses for individual women during the 3rd trimester were 30 to 95 mg/day with standard deviations of 0 to 26.3 mg with an average variation of 9.0 mg/day.

Maternal Hair

Women had a median of 4 (range 1–9) hair samples collected throughout pregnancy. Hair samples were collected at enrollment and approximately once each month (mean time between sample collections, 33 ± 15.2 days) throughout pregnancy; however, time between sample collections ranged from 8 to 91 days.

Mean concentrations of methadone and EDDP in maternal washed proximal segments in the 2nd and 3rd trimesters grouped by cumulative dose ranges are included in Table 1. EMDP was only occasionally present, mainly in specimens with methadone concentrations above the ULOQ. Due to later study enrollment and incomplete dosing records corresponding to collected hair, some women's ($N=10$) hair concentrations could not be included in Table 1. Methadone hair concentrations were $>$ ULOQ in 10 segments from 6 women in the 3rd trimester, and 3 segments from 2 women in the 2nd trimester. Methadone hair concentrations in the 3rd trimester were normally distributed, skewed right with no outlier concentrations for EMDP, and skewed right with 1 outlier (44.7 ng/mg, Z-score 3.84) for EDDP. One woman's methadone concentration in the proximal segment was above the ULOQ in 4 consecutive specimens spanning the 2nd and 3rd trimester; this woman also had the outlier EDDP concentration, 44.7 ng/mg (see Figure 2c and 2d, Subject H). Second trimester hair concentrations were normally distributed for methadone and skewed right with no outliers for EDDP and EMDP.

Washed methadone ($n=83$) and EDDP ($n=61$, ND in 22) hair concentrations were not significantly different from those of unwashed hair (methadone, $P=0.6361$; EDDP, $P=0.8337$). Mean differences in washed and unwashed methadone and EDDP concentrations were 1.4 ± 2.7 ng/mg and 0.12 ± 1.1 ng/mg, respectively.

Distal segments had lower analyte concentrations than proximal segments. Significant decreases were seen for methadone and EDDP concentrations between proximal (segment 1) and more distal segments (segments 2 and 3). Methadone concentration differences between segments 1 and 2 (mean difference 8.9 ng/mg), 1 and 3 (mean difference 10.7 ng/mg) and 2 and 3 (mean difference 2.06 ng/mg) were significantly different in matched paired t-tests (respectively, $P<0.0001$ $n=80$, $P<0.0001$ $n=65$, and $P<0.0001$ $n=60$). EDDP concentration differences between segments 1 and 2 (mean difference 2.4 ng/mg) and segments 1 and 3 (mean difference 3.2 ng/mg) also were significantly different in matched paired t-tests

(respectively, $P < 0.0001$ $n=61$, and $P=0.0006$, $n=35$). EMDP was detected in only 2 distal segments, segments 2 and 3 from one participant; both distal segments had methadone above the ULOQ.

Dose-Concentration Relationship Assessment

Methadone and metabolite dose-concentration relationships were assessed in maternal hair with data from the most proximal maternal hair segment. Maternal cumulative methadone dose was determined for two hair growth rates (1.0 or 1.4 cm/month),^{17–21} a 7-day delay for drug incorporated into the hair root to reach the scalp and be accessible for sampling,^{22–23} and the average length of hair left behind on the scalp after sampling (0.8 cm).¹⁸ For example, in a 3 cm segment, a 1.4 cm/month growth rate is equivalent to a 65-day dosing period and with a 1.0 cm/month growth rate, a 90-day dosing period. Eight unique dosing period variations were tested (Figure 1): with a 1.4 cm/month growth rate, a 65 day dosing period was evaluated beginning 1) on the day of collection (DOC), 2) 7 days prior to DOC (delay for drug to reach scalp), 3) 18 days prior to DOC (0.8 cm left on scalp), and 4) 25 days prior to DOC (combination of the two previous factors). Assuming a 1.0 cm/month growth rate, a 90 day dosing period was evaluated in the same manner.

Testing all factors explained above, no significant linear relationship was seen between cumulative maternal methadone dose and methadone, EDDP or total hair concentrations among all women (Figure 2a, c, and e). Total hair concentrations were determined from the summation of methadone and EDDP; EMDP concentrations were not included because there were few positive specimens, and when present, methadone concentrations were $> \text{ULOQ}$. No dose-concentration relationship was observed for methadone, EDDP or total concentrations. However, within individuals, positive trends were seen between cumulative methadone dose and methadone, EDDP and total concentrations in hair (Figure 2b, d, and f).

Dose-Concentration Ratio Relationships

Positive linear trends were seen for cumulative maternal methadone dose and EDDP/methadone ratio under all tested assumptions. Some linear relationships were significant ($P < 0.05$) prior to considering our Bonferroni adjusted P value ($P=0.00625$). Significant trends were seen with a 1.4 cm/month growth rate, when accounting for 0.8 cm left on the scalp, and the combination of this factor and a 7 day delay for drug to reach the scalp (Figure 1a, black solid and gray dashed lines, $P=0.0094$ and $P=0.0070$, respectively, and Figure 3a). With a 1.0 cm/month growth rate, findings were significant when accounting for growth rate alone, and a 7 day delay in drug to reach scalp (Figure 1b, black solid and gray solid lines, $P=0.0166$ and $P=0.0187$, respectively, and Figure 3b). An outlier EDDP/methadone ratio of 0.73 was excluded (Z -score 6.15) from all dose-concentration ratio analyses; the hair specimen contained 21.6 ng/mg EDDP and 29.4 ng/mg methadone.

Infant Hair and Neonatal Outcomes

Hair was only available from 4 infants; hair from other infants was not collected because there was an inadequate amount or the mother objected to collection. Figure 4 shows that maternal methadone hair concentrations were higher than those in infant hair, although EDDP concentrations were higher in infant than those in maternal hair. Infant EDDP/methadone ratios were higher than maternal hair ratios. EMDP was not detected in any infant hair samples.

Neonatal outcome data were available for 24 infants. At birth, NAS scores ranged from 0 to 11 with a median of 4. Peak NAS scores ranged from 3–18, with a median peak score of 9. Median time to peak NAS was 4 days with a range of 0–11 days. Two infants were not included as they had peak NAS scores of < 4 . Time to NAS onset ranged from 0 to 4 days,

with a median time to onset of 10.7 h after birth. For many infants (n=10), NAS onset began immediately at birth, but for the majority (64%, n=14) of infants, NAS onset began within the first 24 h of life. Duration of NAS ranged from 1 to 19 days, with a median duration of 5 days.

Maternal methadone and EDDP hair concentrations and EDDP/methadone ratios in the proximal segment collected closest to delivery were not significantly correlated with infant NAS score at birth, time to NAS onset, duration of NAS, peak NAS score, time to peak score, neonatal birth length, weight or head circumference ($P>0.05$), although a positive trend was seen for maternal methadone hair concentrations and duration of NAS ($P=0.0502$, $r=0.4680$). Cumulative maternal methadone dose in the 3rd trimester was significantly correlated with birth weight ($P=0.0416$, $r=0.3898$) and a trend was seen with birth length ($P=0.0544$, $r=0.3375$). Cumulative 3rd trimester dose was not significantly correlated with other infant outcomes.

Median morphine administered to infants was 1.7 mg (range 0.6–8.6). Maternal cumulative 3rd trimester methadone dose, methadone, EDDP and EDDP/methadone hair concentrations, neonatal birth length, weight and head circumference were not correlated with amount of morphine pharmacotherapy administered to infants ($P>0.05$).

DISCUSSION

The positive linear trend for cumulative methadone dose and EDDP/methadone ratio in maternal hair (Figure 3) may reflect methadone's induction of its own metabolism^{29–30} or metabolic changes during pregnancy.^{31–33} Methadone is metabolized by several cytochrome P450 (CYP) enzymes including CYPs 3A4, 2D6, 1A2, 2B6 and 2C19, with 3A4 being the most important.³⁴ Methadone metabolic auto-induction was suggested, as decreasing serum concentrations were observed in patients maintained on stable methadone doses.^{29–30} Drug metabolism changes during pregnancy also may be responsible. During pregnancy, metabolic activity of CYPs 3A4 and 2D6 increase, whereas activity of CYPs 1A2 and 2C19 decrease.³² Previous studies showed that methadone elimination was greater during pregnancy^{31, 33} and oral clearance of methadone was significantly increased³¹ suggesting elevated methadone metabolism during pregnancy.

Infant hair EDDP concentrations were higher than maternal EDDP hair concentrations. To our knowledge, this is the first comparison of EDDP concentrations in hair from mother-infant pairs. Placental metabolism of methadone to EDDP by CYP19 increases fetal EDDP exposure, possibly accounting for the higher EDDP concentrations seen in infant hair noted in our population.³⁵ Fetal methadone metabolic rates are unknown, but may play a role. Bidirectional transfer of methadone across the placenta also could be important. Greater methadone fetal to maternal transfer rates were documented than maternal to fetal rates in ex vivo investigations.³⁶ Fetal methadone to EDDP metabolism and EDDP excretion into fetal urine that accumulates in the amniotic fluid also might contribute to the high EDDP concentrations in infant hair.³⁷ Infant hair is continually exposed to drug in amniotic fluid, and specimens were not washed prior to analysis. Consistent with previous publications,^{10, 38–39} EMDP was not found in infant hair, and only occasionally in maternal hair.

Decreased maternal methadone and EDDP concentrations in distal hair segments may be explained by lower methadone doses earlier in pregnancy, and/or damage to the hair cuticle from cosmetic treatments and normal hygienic practices.⁴⁰ In our study, the decrease in methadone and EDDP hair concentrations from the first to the second segment was greater than the decrease from the second to the 3rd segment. This suggests that hygienic processes

remove most of the loosely bound drug initially with less effect over time. A previous study documented decreased concentrations of methadone and EDDP in distal hair segments (1 cm) collected from non-pregnant adult patients participating in a methadone maintenance program for at least a year, although patients' methadone doses were not described.¹⁵ The decrease in EDDP/methadone ratios in distal segments indicates that EDDP may be lost from hair more readily than methadone, possibly due to stronger binding of the basic parent drug to melanin in hair. An additional possibility for this decrease in distal segments may be limited cytochrome P450 auto induction at lower methadone concentrations earlier in pregnancy.^{29–30}

Our study was unable to demonstrate a statistically significant dose-concentration relationship for methadone dose and methadone, EDDP or total concentrations in hair of methadone-assisted pregnant women. There was large inter-subject variability; relationships improved when isolating data from individual women (Figure 2b). Possible confounding factors not assessed were changes in methadone metabolism during gestation, hair growth differences specific to different stages of pregnancy, hair melanin content, and drug disposition in sweat that might also change during pregnancy.^{24, 32–33, 41} Other limitations include single SRM for identification, and lack of quantitative results for samples with methadone concentrations above the ULOQ (50ng/g).

The positive correlation between cumulative maternal methadone dose in the 3rd trimester and infant birth weight suggests healthier pregnancies for women receiving adequate methadone pharmacotherapy. Others also documented increased birth weight or length with higher methadone doses during pregnancy.^{42–43} Higher maternal methadone dosing may generally reduce heroin relapse and reflect a less stressful lifestyle and better nutrition, resulting in healthier fetal weight gain.⁴⁴ Previous studies demonstrated that past heroin abuse, despite abstinence during pregnancy, could contribute to reduced birth weights,⁴⁴ suggesting that heroin induced maternal physiologic changes.⁴⁵ Methadone may correct for reductions in fetal organ cell numbers⁴⁴ or brain size seen in neonates born to heroin-addicted mothers.⁴

We did not document the predictive value of maternal EDDP/methadone hair concentration ratio, dose or maternal and infant methadone or EDDP hair concentrations for peak NAS scores, time to peak scores, time to NAS onset, duration of NAS or amount of required morphine pharmacotherapy. We previously noted that placental methadone and EDDP concentrations correlated with maternal methadone dose at delivery, mean daily dose and cumulative 3rd trimester dose.⁴⁶ Umbilical cord methadone concentrations were significantly correlated with maternal daily and 3rd trimester methadone dose, and umbilical cord EDDP concentrations and EDDP/methadone ratios correlated with neonate birth length, peak NAS score, and time to peak NAS score.⁴² However, multiple investigators failed to document a significant relationship between infant NAS scores and maternal methadone dose, and methadone concentrations in breast milk, maternal or infant plasma, amniotic fluid, meconium and fetal urine.^{47–51}

CONCLUSION

Our data suggests it is not possible to estimate the degree of methadone exposure during pregnancy based on maternal hair concentrations. This is important for clinical interpretation of hair test results. Methadone assisted therapy during pregnancy provided a controlled drug administration model for studying drug disposition in the hair of opioid dependent pregnant women and their infants. Although maternal and neonatal hair were good matrices for documenting methadone exposure, methadone and EDDP hair concentrations did not predict

the magnitude of methadone exposure or dose, nor the probability of adverse neonatal outcomes.

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Cumulative Dose Calculations

a) Accounting for a 1.4 cm/month growth rate

b) Accounting for a 1.0 cm/month growth rate

FIGURE 1.

Linear representation of tested dosing periods for the 3 cm proximal maternal hair segment under two hair growth rate assumptions, a) 1.4 cm/month and b) 1.0 cm/month. A 3 cm segment with a hair growth rate of 1.4 cm/month corresponds to 65 days of maternal methadone dosing, while a 1.0 cm/month hair growth rate corresponds to 90 days of dosing. Further variability is introduced by the delay for hair with incorporated drug to reach the scalp, and hair left on the scalp when sampling with scissors. Black solid lines below the timeline represent the dosing periods under only the growth rate assumptions. Gray solid lines represent dosing periods accounting for a 7 day delay in drug incorporated to reach scalp level. Black dashed lines indicate dosing periods accounting for 0.8 cm (18 days, or 24 days depending on the growth rate) of hair left behind on scalp when sampling. Gray dashed lines account for both a 7 day delay and 0.8 cm left behind on scalp.

FIGURE 2.

Comparison of dose-concentration relationships for methadone (a), EDDP (c) and total (e) hair concentrations under the assumption of a 1.4 cm/month hair growth rate. Individual dose-concentration relationships for methadone (b), EDDP (d) and total (f) hair concentrations for 7 women; only women with 3 or more hair collections and corresponding dosing information were included. Subject H is excluded from all graphs except (d) as methadone concentrations were above the ULOQ in all hair specimens.

a) Metabolite/Parent Drug Ratio in Hair (1.4 cm/month Growth Rate)

b) Metabolite/Parent Drug Ratio in Hair (1.0 cm/month Growth Rate)

FIGURE 3.

A positive linear trend between maternal cumulative dose and metabolite/parent (EDDP/methadone) concentration ratio in proximal maternal hair segments. This relationship is significant without adjusting for multiple comparisons. Data shown in a) assumes a 1.4 cm/month hair growth rate, a 7 day incorporation delay and 0.8 cm hair left behind when sampling; $y = (2.48 \times 10^{-5})x + 0.0045$. Data in b) assumes a 1.0 cm/month growth rate and a 7 day incorporation delay; $y = (1.70 \times 10^{-5})x + 0.013$.

FIGURE 4.

Maternal methadone hair concentrations (black bars, top) were higher than those in paired infant hair (white bars, top), although EDDP concentrations were higher in infant hair (black bars, bottom) than in maternal hair (white bars, bottom).

Summary of Methadone and Metabolite Hair Concentrations from Opioid-Dependent Pregnant Women Grouped by Cumulative Methadone Dose

TABLE 1

3 rd Trimester					
Cumulative Methadone Dose (mg)	Daily Methadone Dose (mg)	Number of Women	Number of Hair Samples	Methadone Hair Concentrations (ng/mg) Mean $\pm$ SD	EDDP Hair Concentrations (ng/mg) Mean $\pm$ SD
2500–3990	45–60	3	3	29.4 ^a	16.2 $\pm$ 7.6
4000–4990	65–75	8	9	17.3 ^a $\pm$ 9.7	4.5 ^b $\pm$ 3.6
5000–5990	75–90	5	8	28.1 ^a $\pm$ 15.4	5.3 $\pm$ 2.1
6000–6990	95–100	2	3	21.9 $\pm$ 4.4	3.2 $\pm$ 1.3
2 nd Trimester					
Cumulative Methadone Dose (mg)	Daily Methadone Dose (mg)	Number of Women	Number of Hair Samples	Methadone Hair Concentrations (ng/mg) Mean $\pm$ SD	EDDP Hair Concentrations (ng/mg) Mean $\pm$ SD
2500–3990	45–60	6	9	24.3 $\pm$ 10.2	1.5 $\pm$ 0.7
4000–4990	65–75	6	9	19.7 ^a $\pm$ 9.4	3.9 $\pm$ 2.3
5000–5990	75–90	1	1	20.6	1.9
					ND

^aConcentrations above the ULQ were excluded as reanalysis was not performed

^bOutlier EDDP concentration (44.7ng/mg) excluded

^cND: Not detected

^dIn all except 2–3 samples in each dose category, EMDP was ND; therefore, median is shown in place of mean