Evaluation of Maternal Toxicity in CF-1 Mice Following Gestational Opioid Exposure

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Abstract

The risks associated with opioid exposure during the first trimester of pregnancy are not fully understood. Limitations with previous epidemiological study designs, conflicting results from human and animal studies, and incomplete maternal toxicity data complicate risk assessment. To address the maternal toxicity data gap, CF-1 mice were treated subcutaneously with morphine [100 or 400 mg/kg] or methadone [10 or 30 mg/kg] on gestational day (GD) 8 of pregnancy (plug date = GD 0) and assessed for signs of hypoxia. The known human teratogen, valproic acid (VPA) [300 or 500 mg/kg], was used as a positive control compound. The Vevo 3100 preclinical imaging system (FUJIFILM Visual Sonics), with color and pulse doppler, was used to measure uterine artery blood-flow 30 minutes following drug treatment (n=10/dose). Uterine artery velocity (UAV), pulsatility index (PI) and resistance index (RI) were then calculated. UAV in all treatment groups was higher than control values, while PI and RI were lower in the morphine and methadone groups. The observed changes, while indicative of hypoxia, were not dose-dependent or significantly different from controls in a one-way ANOVA with Tukey's post-hoc analysis. The Element POC (Heska) was used for blood gas measurements. Oxygenated blood samples, collected from the left ventricle, were assessed at 30 minutes and 2.5 hours post-dosing (n=10/dose/timepoint). Statistically significant differences at 30 minutes included: lower blood pH, increased partial pressure of carbon dioxide (pCO2), bicarbonate and total carbon dioxide (TCO2) levels. Changes were observed in both the low and high dose opioid treatment groups. At 2.5 hours post-dosing, the pH and pCO2 levels remained significantly different. While decreases in the partial pressure of oxygen (pO2) were anticipated, the only statistically significant decrease occurred in the methadone 30 mg/kg group. VPA did not affect clinical blood gas parameters in mice. Changes in blood gas measurements, combined with the uterine artery blood flow data, indicate that opioid exposure induces maternal hypoxia in CF-1 mice. The data presented here will be combined with teratological assessments and maternal and fetal toxicokinetic data to provide a more comprehensive understanding of the risks related to opioid-exposure during early pregnancy.

Introduction

- In a 2015 Drug Safety Communication, the FDA announced a review of recent studies suggesting an increased risk of neural tube defects (NTDs) when exposure to opioids occurred during the first trimester of pregnancy.
- Limitations in the study designs, however, prevented the FDA form updating recommendations for opioid use during pregnancy.
- Conflicting results from human and animal studies and incomplete maternal toxicity data further necessitates additional studies to evaluate maternal toxicity following gestational opioid exposure.

Materials and Methods

- Time-mated female CF-1 mice were obtained from Charles River Laboratories (plug day = GD 0). Mice arrived at NCTR on GD 4 and were randomly assigned to one of the seven treatment groups (n=10/dose).
- On GD 8 mice were injected subcutaneously with a single injection of vehicle [0.9 % saline], morphine [100 or 400 mg/kg], methadone [10 or 30 mg/kg], or VPA [300 or 500 mg/kg].
- Uterine artery flow measurements, recorded 30 mins after dosing, the Vevo 3100 preclinical imaging system (Fujifilm Visual Sonic) with color and pulse doppler was used to calculate uterine artery velocity (UAV), pulsatility index (PI), and resistance index (RI).
- Blood gas measurements were recorded 30 minutes and 2.5 hours post-dosing (n=10/dose/timepoint). Oxygenated blood from the left ventricle was obtained and the Element POC (Heska) was used for measurement.
- A one-way ANOVA with Tukey's post-hoc analysis was used to determine statistically significant differences among treatment groups. Statistical significance was set at $p \leq 0.05$.

Results

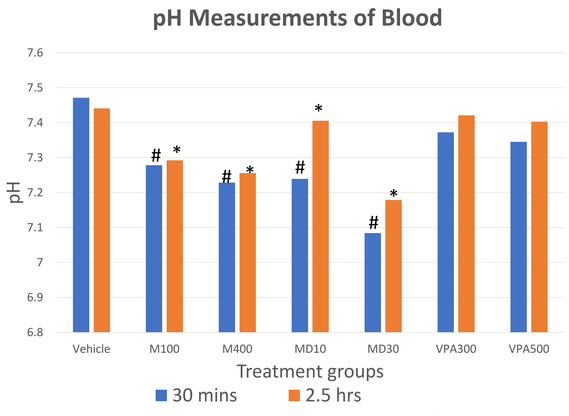

Figure 1. pH measurements on oxygenated blood at 30 minutes and 2.5 hours after opioid or VPA treatment in CF-1 mice on GD 8. Statistically significant differences were observed following exposure to morphine or methadone, but not following VPA treatment. # and * indicate statistically significant differences from controls at 30 minutes and 2.5 hours, respectively.

Results Continued

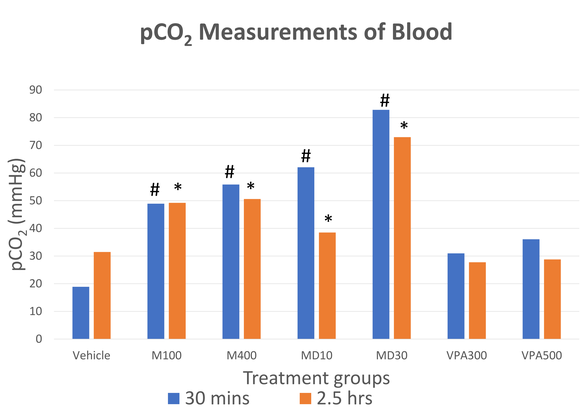

Figure 2. pCO_2 measurements on oxygenated blood at 30 minutes and 2.5 hours after treatment in CF-1 mice on GD 8. pCO_2 levels were statistically different from controls at 30 min. for both morphine and methadone and remained elevated at 2.5 hrs. for all opioid groups except low dose methadone. # and * indicate statistically significant differences from controls at 30 min. and 2.5 hrs., respectively.

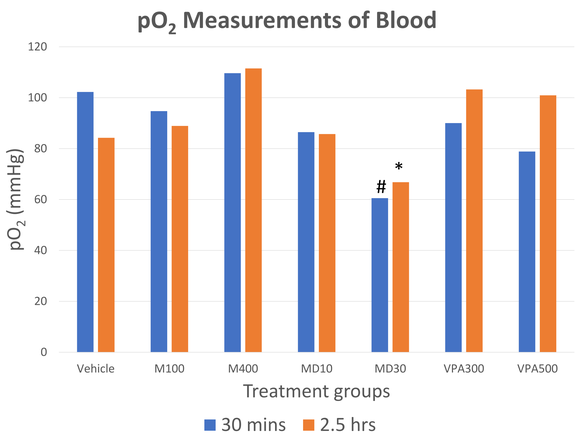

Figure 3. pO_2 measurements on oxygenated blood at 30 minutes and 2.5 hours after treatment in CF-1 mice on GD 8. pO_2 levels were statistically different from controls at 30 min. for methadone high dose and remained elevated at 2.5 hours. # and * indicate statistically significant differences from controls at 30 minutes and 2.5 hours, respectively.

Uterine Artery Flow Measurement in CF-1 mice on GD 8

Group	UAV (mm/s)	UA RI	UA PI	No. of Dams	NTD (F/D)
Vehicle	237 (±88.15)	0.6273 (±0.09)	1.1783 (±0.37)	9	
M-100	318.5 (±188.36)	0.4532 (±0.18)	0.7737 (±0.51)	9	
M-400	388.1 (±241.74)	0.5448 (±0.12)	1.0362 (±0.36)	8	9/2
MD-10	205.5 (±58.69)	0.5682 (±0.09)	1.0881 (±0.25)	4	
MD-30	319.69 (±94.12)	0.6179 (±0.09)	0.8855 (±0.41)	5	
VPA-300	379.16 (±208.39)	0.6411 (±0.08)	1.2321 (±0.27)	7	
VPA-500	371.55 (±323.73)	0.6436 (±0.08)	1.2499 (±0.39)	6	6/3

Measurements are reported as the mean (±SD). NTD: Neural tube defects, F/D: total number of fetuses/ total number of dams.

Table 1: Uterine artery velocity (UAV) increased in all treatment groups except for the methadone low dose group, when compared to control. Uterine artery pulsatility index (UA PI) and uterine artery resistance index (UA RI) decreased in morphine and methadone, both low and high dose groups. Neural tube defect (NTD), specifically exencephaly, were noted in the a few fetuses in the morphine and VPA high dose groups upon sacrifice of the dams on GD 18.

Conclusion

- The increased UAV in treatments groups compared to control may indicate maternal toxicity, while decreased UA RI and PI may represent decreased blood flow to fetuses.
- Blood gas analysis with decreased pH and increased pCO_2 are also suggestive of maternal hypoxia.
- Detailed teratological analyses and maternal fetal toxicity data will be combined with the uterine artery blood flow and blood chemistry data to provide a comprehensive analyses of risks associated with opioid exposure in early pregnancy.

Acknowledgement

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