Supplemental Material

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Supplemental Note
Pharmacokinetic Methods and Results

Table S1. Potential confounding factors in primary outcomes in neonates.

Sex of neonates

Primary outcome	**Male (N=45)**	**Female (N=41)**	**P value**
Number of infants requiring morphine to treat NOWS – # (%)	25 (56%)	23 (56%)	1*

*Fishers Exact test.

Table S2. Potential confounding factors in primary outcomes in mothers.

Race/ethnicity of mothers

Primary outcome	**NH/W (N=69)**	**NH/Black (N=10)**	**Hisp/W (N=6)**	**NH/Black + other (N=1)**	**P value**
Need of morphine to treat NOWS – # (%)	39 (57%)	5 (50%)	3 (50%)	1 (100%)	0.80*

*Pearson's Chi-squared test.

Figure S1. Ondansetron Concentration in Infants. The plasma ondansetron concentration [ONDS] was sequentially measured in 14 neonates. The measurements performed before a baby received a dose of ondansetron are labeled as Birth #1 and Birth #2, and reflect placental transfer from the mother. Birth #1 was cord blood and Birth #2 was heel stick. The Dose 1 and Dose 2 measurements were performed after the 1st or 2nd dose of ondansetron, respectively, was administered to the baby.

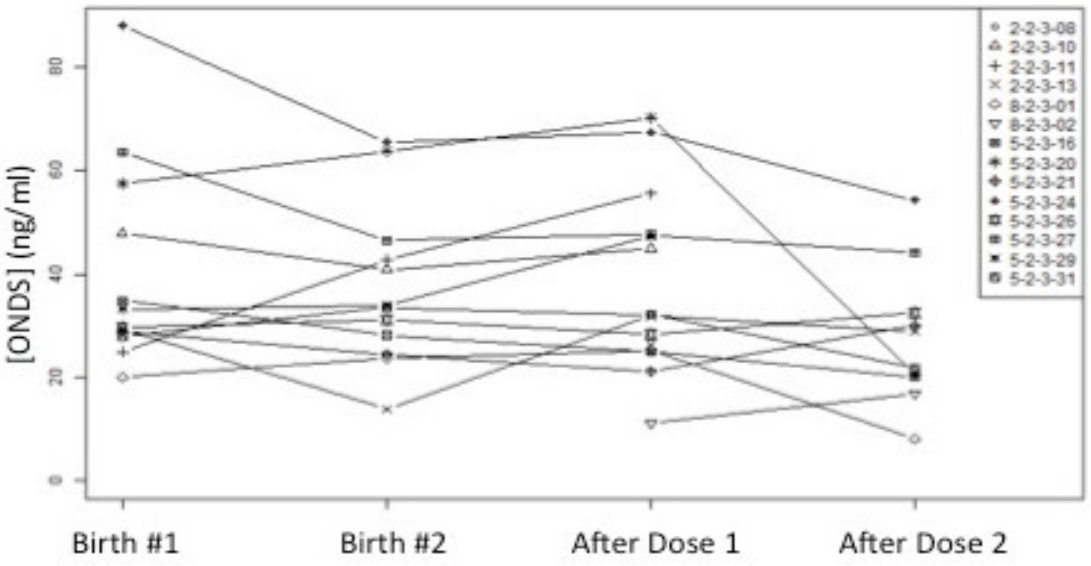
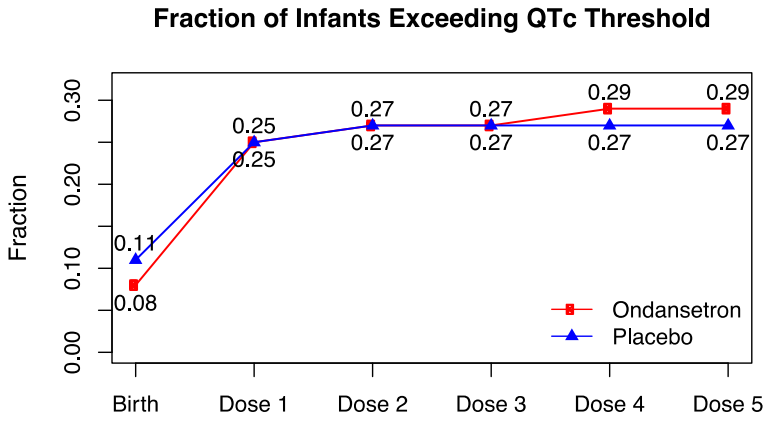


Figure S2. The fraction of infants with the QTc values above the threshold of the screening ECG and after each dose of study drug.



Ondansetron Concentration Measurements

Ondansetron HCl hydrate (Sigma) and the internal standard ondansetron-D3 (Santa Cruz Biotechnology) were used in these analyses. High-performance, liquid-chromatography (HPLC)-grade water, methanol, and acetonitrile, which were used for sample extraction and as the mobile phase. The calibration curve ranged from 219.6 to 0.429 ng/ml. Three quality control samples were also prepared at concentrations of 109.8, 10.98 and 1.098 ng/ml. Plasma samples were mixed with 3 volumes of acetonitrile containing the internal standard, dried and reconstituted in 50 ul of 0.1% formic acid, 5% acetonitrile in HPLC water. Dried blood spot samples were used for ondansetron measurements in neonates.[1] The calibration curve range for ondansetron ranged from 219.6 ng/mL to 0.429 ng/mL. The extracts were analyzed using an LCMS system according to previously described methods.[2] Briefly, 10 µL of reconstituted supernatant for plasma samples or 18 µL for dried blood spot samples was injected onto an analytical column (Phenomenex) C18 2.6 u 100 x 2.1 mm. A gradient was run from 95% solvent A (0.1% formic acid in water) to 95% solvent B (0.1 % formic acid in acetonitrile) over 30 min at solvent B: flow rate of 0.5 mL/min. An Agilent 1290 Infinity UHPLC system was interfaced with an Agilent QTOF accurate mass 6520 mass spectrometer using a positive electrospray ionization source. Full scan (m/z 110–1000) spectra were collected. For ondansetron, the ion was monitored: 294.1601 $[M+H]^+$. For the ondansetron D3 internal standard, the ion was monitored: 297.1789 $[M+H]^+$.

Ondansetron concentration in neonates

Our ondansetron dosing regimen was based upon results obtained from a prior population pharmacokinetic analysis.[3] To assess ondansetron exposure in the treated neonates, plasma ondansetron concentrations were measured in 14 neonates that received ondansetron and had a blood drawn for purposes unrelated to the study. The interval between ondansetron dosing and the sample acquisition time varied because sample acquisition was only obtained when blood was required for other purposes**.** Nevertheless, the plasma ondansetron concentrations measured after birth (but before the baby received a dose of ondansetron) averaged 41 $\pm$ 20 ng/ml (range 20 to 88 ng/ml) and after receiving one or more ondansetron doses averaged 38 $\pm$ 18 ng/ml (range 11 to 77 ng/ml) (**Fig S1**). Of note, the ondansetron concentrations in the neonates were very similar to those measured in non-pregnant women (mean 33 ng/ml) and in pregnant mothers (mean 46 ng/ml) in our prior pharmacokinetic study,[3] and are within the therapeutic range found after oral ondansetron dosing in several different adult populations.[4] Thus, this dosing regimen produced plasma ondansetron concentrations required for anti-emetic efficacy.

1. Xu X, Bartlett MG, Stewart JT. Determination of ondansetron and its hydroxy metabolites in human serum using solid-phase extraction and liquid chromatography/positive ion electrospray tandem mass spectrometry. J Mass Spectrom. 2000;35(11):1329-1334.

2. Spooner N, Lad R, Barfield M. Dried blood spots as a sample collection technique for the determination of pharmacokinetics in clinical studies: Considerations for the validation of a quantitative bioanalytical method. Anal Chem. 2009;81(4):1557-1563.

3. Elkomy MH, Sultan P, Carvalho B, et al. Ondansetron pharmacokinetics in pregnant women and neonates: Towards a new treatment for neonatal abstinence syndrome. Clin Pharmacol Ther. 2015;97(2):167-176.

4. Roila F, Del Favero A. Ondansetron clinical pharmacokinetics. Clin Pharmacokinet. 1995;29(2):95-109.