Pharmacokinetics and Pharmacodynamic Data Analysis Plan PART 1

For

PROTOCOL: NCT04037085 and FDAAA801

STUDY TITLE:

Ketamine to Improve Recovery After Cesarean Delivery – Part 1

(KINETIC)

VERSION 2.0

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I. Clinical Protocol

In a prospective, open label, observational design study, women who were weaning from breastfeeding were enrolled. Women were included if they were ≥18 years of age, able to provide informed consent, weaning from breastfeeding, ASA physical status 1, 2, or 3, and producing a minimum of 2mL breast milk every 24 hours. They were excluded if they were allergic to study medications, ASA physical status 4 or greater, had a history of psychiatric comorbidity, uncontrolled hyperthyroidism, cardiac disease, fever, hypertension, history of hallucinations, alcohol, or illicit substance abuse, were on chronic opioid therapy, or had chronic pain disorders.

II. Dose Administration

Subjects received a sub-anesthetic ketamine infusion at 0.1 mg/kg/hour for 12 hours (not to exceed 8mg/hr dose). The justification for intravenous administration for 12 hours of 0.1mg/kg/hr are based on pharmacokinetic studies in humans[18-20]. Ketamine produces analgesia at plasma concentrations of 100-200ng/mL, which is a fraction of the plasma concentrations associated with general anesthetic doses (9000-25,000 ng/mL)[18, 19]. In longitudinal studies reporting ketamine infusions from 0.05 -1mg/kg/hr, significant reductions in pain scores with minimal adverse events and no psychomimetic effects were observed over infusions lasting up to 5 days, while the effect of ketamine on pain scores were notably varied according to infusion duration[21, 22].

III. PK Sampling Schedule of Treatment Group

Blood samples (2-3mL each) were collected at 8, 10, 12, 12.5, 13, 14, 16, and 20 hours after ketamine infusion start. Plasma was obtained by centrifugation at room temperature and kept frozen until analysis. The entire human milk expressed was collected starting at the time of infusion initiation (t_0) and ending 12 hours after the infusion was stopped (t_{24}). Breastmilk was labeled by collection time and weighed to estimate the volume (1 gram = 1 mL) and stored at -80ºC until analysis. Patient vital signs were monitored every hour and continuous pulse oximetry was done for 12 hours during the infusion and for 12 hours after infusion.

IV. Analytic Methods: Liquid Chromatography-tandem Mass Spectrometry (LCMS/MS) Assay for Ketamine and Metabolites in Breast

Samples were separated using a Waters UPLC equipped with Acquity UPLC BEH RP18 1.7µm, 2.1 × 100 mm column combined with a guard filter (Waters # 289002078). The Liquid Chromatography (LC) method consisted of a gradient mobile phase of Solvent A (95% 2mM Ammonium Acetate + 5%

Acetonitrile + 0.1 % Formic Acid) and Solvent B (95% Acetonitrile and 5% Water) for elution of the analyte. The gradient started at 100% of Solvent A which was held for 1.0 minute and then changed to 80% Solvent A and 20% Solvent B which was kept constant for the next 3 minutes. The solvent composition then changed to 65% Solvent A and 35% Solvent B over 0.1 min and then immediately to 30% Solvent A and 70% Solvent B which was maintained for 1 minute. The gradient then returned to the initial solvent condition of 100% Solvent A, achieving the initial base line. The total run time was 7 minutes with a flow rate of 0.3 mL/min.

Analysis was performed on a Waters TQ-S Mass Spectrometer (Waters, Milford, MA) in positive electron spray mode (+ESI) using multiple reaction monitoring (MRM). The following MRM settings were employed: Capillary Voltage 1.5kV, de-solvation temperature 500°C, Cone gas flow 150 L/h, de-solvation gas flow 1000 L/h; Argon pressure 0.15 mL/min, Nitrogen pressure 7 bar and dwell time was 0.038 sec. The extracted ions following MRM transitions monitored were m/z 237.84→124.994 for KET, m/z 223.9→125.01 for NKET, m/z 221.9→141.83 for DHNK, m/z 241.9→128.91 for KET-D_4 (IS for KET and DHNK) and m/z 227.9→128.96 for NKET-D_4 (IS for NKET). The LC and mass spectrometer were controlled, and data collected using Masslynx® software version 4.2 (Waters Corporation, Milford MA).

Ketamine (KET) and its metabolites (NKET & DHNK) were analyzed in plasma and breast milk samples using ultra high-pressure liquid chromatography and mass spectrometric analysis. The developed assay was linear between 0.05 - 100 ng/ml and Coefficient of Variability (CV) of the assay was less than 10%.

V. PK Calculations

The pharmacokinetic parameters were calculated using Phoenix™ WinNonlin 8.3.4 (Certara, Princeton NJ). A two compartment PK model was used to estimate PK parameters of ketamine. These parameters included Area Under the Curve (AUC) estimates, the elimination half- life ($t_{½}$), clearance (CL), Area Under Moment Curve (AUMC), Mean Residence Time (MRT) and Volume Distribution Steady State (V_{dss}). Statistics will include Mean, Standard deviation (SD), and range where applicable.

a. **Area Under the Curve (AUC)** is defined as the area under the concentration-time curve. AUC was calculated by linear trapezoidal method.
b. **Elimination half-life ($t_{½}$)** is the time required for drug concentration to decrease by one-half at the end drug dosing. Elimination half-life was obtained from the slope of terminal elimination phase.
c. **Clearance (CL)** is calculated as the Dose/$AUC_{0-\infty}$ and calculated by Phoenix WinNonlin.

d. **Volume Distribution Steady State (Vdss)** is the period of dynamic equilibrium of the drug calculated as the amount of drug in the body at time, t divided by the plasma concentration of the drug at time, t. These data were calculated by Phoenix WinNonlin.
e. **Metabolic ratio** was calculated for each metabolite using the formula:

$$Metabolic\ ratio = \frac{Metabolite\ AUC_{0-\infty}}{Parent\ AUC_{0-\infty}}$$

VI. Milk to Plasma Ratio

Milk to plasma ratio for KET, NKET, and DHNK were calculated by dividing the concentration of the respective components Ketamine and Ketamine metabolites in human milk by plasma concentration at the corresponding times (± 30 min). Ratios higher than 1 indicate breastmilk concentrations of ketamine and the metabolites would be higher in breastmilk than in maternal plasma concentrations.

VII. Relative Infant Dose (RID) Calculations

The relative infant dose was calculated from the concentrations of ketamine and its metabolites in breast milk at different times following ketamine administration to the women. The concentration of ketamine and its metabolites were converted to amount by multiplying with the volume of breast milk collected at various time intervals. The cumulative dose of ketamine and molar equivalents of metabolites was calculated and RID was calculated using the formula [23-25]: An RID ≤10% was considered low[24].

$$RID\ (\%) = Cumulative\ infant\ dose\ via\ breast\ milk \left(\frac{\frac{mg}{kg}}{day}\right) X\ 100 \div maternal\ dose \left(\frac{\frac{mg}{kg}}{day}\right)$$

VIII. Descriptive Statistics

Descriptive Statistics will include participant characteristics such as maternal age, maternal race, gravida, parity, ASA rating, and weight. Means and standard deviations (SD) are calculated for maternal age and weight. Medians and range are used for maternal gravida, parity, and ASA rating. Maternal race is noted as a proportion of the total sample.