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Evaluation of early fetal exposure to vaginally-administered metronidazole in pregnant cynomolgus monkeys

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ABSTRACT

Given concern about potential embryo-fetal harm following seminal exposure to drugs with teratogenic potential, pharmaceutical companies use theoretical calculations to estimate seminal concentrations, maternal exposure, and distribution across the placenta to the embryo-fetal compartment for risk assessment. However, it is plausible that there are additional mechanisms whereby the conceptus is exposed. In order to determine if theoretical calculations are sufficiently conservative to predict embryo-fetal exposure from drugs in semen, pregnant cynomolgus monkeys were given a vaginal dose of metronidazole during the early fetal period and cesarean-sectioned. Maternal, fetal, and amniotic fluid samples were analyzed for metronidazole and 2-hydroxymetronidazole. Exposure to metronidazole and its metabolite were comparable in all matrices. These data demonstrated no preferential transfer mechanism to conceptus following intravaginal administration of a small molecule drug; and therefore, suggest that traditional modeling for embryo-fetal exposure to drugs in semen in support of risk assessment for pharmaceutical agents is sufficiently conservative.

1. Introduction

In traditional pharmaceutical development, ICH Harmonized Tripartite Guideline embryo-fetal development studies in animals are conducted to support the entry of women of childbearing potential into clinical trials, and provide guidance for the women taking pharmaceutical products during pregnancy (both intentionally or non-intentionally, in the event that the pregnancy was not planned or recognized) [1,2]. Based on the developmental toxicity profile generated by nonclinical evaluation of treated maternal animals and their conceptuses, pregnancy labels generally focus on potential implications to the embryo-fetus related to use of drugs in women.

However, exposures to the conceptus are possible in pregnant partners of men who are treated with drugs. Small molecule medicines are known to distribute to semen, and in some limited cases accumulate in this compartment. As reviewed by Pichini et al. [3], Kashuba et al. [4], and Klemmt and Scialli [5], the semen-to-blood ratio of over 50 drugs was typically less than or equivalent to 1, i.e., concentrations in seminal fluid did not exceed those in the systemic circulation. However, there were instances whereby higher ratios were identified (typically associated with antimicrobials), with accumulation in semen up to 11.3× that in circulation [6]. As such, there is potential concern that pregnant partners of men treated with an agent with teratogenic liabilities identified at exposures less than or equivalent to clinical levels, or associated with mechanism of actions related to development, could adversely affect the developing conceptus, due to exposure to drug in semen.

To investigate the likely manifestation of these theoretical concerns for paternally-mediated developmental toxicity, typically companies conduct a theoretical risk assessment using a series of assumptions to predict a "worst case" exposure to a developing conceptus and make risk assessments. In this approach, using the upper range of typical human semen volume, an accumulation of drug in semen of 10-fold, complete absorption from the vaginal compartment into maternal circulation, and then finally distribution of the drug throughout the maternal depots, including the conceptus, a predicted maternal systemic exposure is estimated. However, due to the relatively small seminal volume and dilution of the seminal dose in maternal circulation, the likely maternal exposure to drugs

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in semen is several orders of magnitude less than those in male patients directly taking the drug, limiting the concern for adverse paternally-mediated effects to only the most potent of teratogens [5].

While these conservative assumptions indicate the risk is low, it is recognized there are data gaps for potentially unmodeled exposure to the conceptus because typically, nonclinical animal models are not receptive to mating following onset of pregnancy. As described by Klemmt and Scialli [5], there are hypothetical mechanisms for potential local delivery of drugs in semen to the conceptus including: (1) countercurrent transfer from vaginal veins to the uterine arteries, (2) trans-cervical transport, and (3) adsorption of the drug to sperm (the last relevant to the time of fertilization only). In order to derive animal data to evaluate the potential for delivery of drugs in semen to a conceptus at levels beyond those modeled by theoretical assumptions, this study was designed and conducted to evaluate the exposures to monkey fetuses in early gestation following maternal vaginal administration of the antimicrobial agent, metronidazole. Additionally, this study was designed to contribute to the body of science as part of the Health and Environmental Sciences Institute (HESI) Developmental and Reproductive Toxicology (DART) Drugs in Semen Consortium [7].

2. Materials and methods

2.1. Animals and animal care

Care and use of the animals was in conformity with the American Association for Laboratory Animal Science Policy on the Humane Care and Use of Laboratory Animals. All procedures performed on the animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Charles River Laboratories. Sexually mature and experimentally-naïve Chinese cynomolgus monkeys (*Macaca fascicularis)* were used for this study. Females were naturally-mated for 3 days with a breeder male based on anticipated timing of ovulation; pregnancies were confirmed by ultrasound periodically, and on the morning of experimental treatment. Animals were individually housed in stainless-steel cages with environmental enrichment and grooming bar access to adjacent cages. Rooms were environmentally controlled with an approximate 12-h light/dark cycle and maintained at 64 to 84 °F and 30–70% humidity. Monkeys were provided a rationed amount of Purina certified primate diet (No. 5048) daily, with supplemental fruits and vegetables provided 2–3 times weekly, and municipal water, processed by reverse-osmosis filtration and UV-light treatment, ad libitum.

2.2. Experimental design

Three pregnant female monkeys were used in the study (5.1–5.3 years old), all receiving treatment with a single intravaginal dose (1 mL) of 0.75% metronidazole gel (Sandoz Inc., Princeton, NJ, molecular weight = 171) during the early fetal period [Gestation Day (GD) 60, 70, or 71 for Female Nos. 1502, 1501, and 1503, respectively]. The pharmacokinetics of intravaginal metronidazole gel in humans are well characterized [8]; compared to oral administration, absorption of metronidazole from intravaginal gel is more prolonged, incomplete, and variable with peak systemic exposures observed 6 to 12 h after vaginal dosing. Since the drug is already dissolved in gel, this formulation is easily absorbed as the gel improves contact with the vaginal surfaces relative to tablet or cream applications. Given all of these factors, metronidazole gel was selected for use in this study. Doses were given with a 1 mL syringe, inserted approximately 0.25–0.5 inch into the vagina of chair-restrained pregnant females. The gel was dyed blue with food grade blue dye immediately prior to use to ensure the gel could be visualized for potential leakage. Females were continuously monitored for an hour after dosing to check for expulsion of gel and provided edible enrichment during this time.

2.3. Cesarean-sections and sample collection

Approximately 7 h after dosing (within the range of expected C_{max}), each monkey was anesthetized and a cesarean section was conducted. Immediately prior to surgery, a 1-mL blood sample was collected from the femoral artery of each female. At C-section, a 3-mL sample of amniotic fluid was collected and retained. Following removal of the fetus from the gestational sac, the umbilical cord was clamped and cut 2–3 cm from the umbilicus; umbilical blood was collected. Fetuses were anesthetized with phenytoin and pentobarbital (Beuthanasia®-D) via intraperitoneal injection. Fetal blood collection was attempted by cardiac puncture, decapitation, and/or sampling of the vena cava. Fetal body weights were measured. All maternal and fetal blood samples were collected into tubes with anticoagulant (K_2EDTA) and then processed to plasma. Samples were stored at −80 °C until analyzed. Female monkeys were returned to the facility stock colony following recovery from the surgical procedure.

2.4. Sample concentration bioanalysis

Monkey plasma and amniotic fluid samples were prepared by liquid–liquid extraction in ethyl acetate. Analytes were injected into a Phenomenex MAX RP analytical column and analyzed by Turboionspray Ionization (TIS) tandem mass spectrometry (MS/MS) in the positive ion multiple reaction monitoring mode. The concentrations of metronidazole and the major metabolite, 2-hydroxymetronidazole, in plasma and amniotic fluid quality controls and unknown samples were calculated by weighted linear regression from plasma calibration standards run simultaneously with the quality controls and unknown samples. Data was analyzed with Sciex program Analyst, Version 1.4.1. Overall precision and accuracy for the calibration standards and quality control samples met the established acceptance criteria.

3. Results

Single, fixed volume, vaginal doses of 7.5 mg metronidazole were well tolerated by pregnant cynomolgus monkeys. No clinical observations were noted after dose administration. Leakage of the test article material from the vagina was noted for the first monkey dosed. As such, insertion of the dosing syringe was increased from 0.25 to 0.5 inches into the vagina for the 2 subsequent animals tested. The modified dosing technique eliminated issues related to leakage and loss of the test material.

Fetal blood sample collection was attempted by several routes following euthanasia; the largest blood samples were collected from the umbilical cord or at the site of umbilicus attachment. Collections attempted by cardiac puncture or from the vena cava were unsuccessful. Sufficient blood was obtained from the umbilicus, the umbilical cord, and fetal trunk blood harvested from the fetus on GD 71 (Female No. 1503) such that individual bioanalysis could be conducted on samples from each site (Table 1). Based on the results from this single fetus, the site of blood sampling had no effect on the concentration of metronidazole or the metabolite hydroxymetronidazole; all values were comparable (Fig. 1).

Maternal exposures to metronidazole were variable when assessed 7 h after a single fixed volume vaginal dose (Table 2); individual maternal plasma values ranged from 94 to 756 ng/mL. Relatedly, there were similar variations in the fetal plasma and amniotic fluid concentrations measured for metronidazole

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