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Investigation of maternal and fetal exposure to an IgG2 monoclonal antibody following biweekly intravaginal administration to cynomolgus monkeys throughout pregnancy

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ABSTRACT

To assess the potential for male-mediated drug transfer to their female partner and/or developing conceptus, vaginal uptake of a monoclonal antibody (mAb) biotherapeutic was assessed in cynomolgus monkeys. A human IgG2 mAb (IgG2X; bound human and cynomolgus monkey neonatal Fc-receptor, FcRn, with similar high affinity) was administered intravaginally (IvG; 100 mg/dose) to 5 pregnant cynomolgus monkeys biweekly from gestation day (gd) 21 to gd133. In all maternal samples collected before gd119, IgG2X plasma concentrations were below the limit of quantification (BLQ; <25 ng/mL). After dosing on gd119 and 133, maternal IgG2X plasma concentrations remained BLQ in 3/5 monkeys and were very low in 2/5 (up to 116 ng/mL; ~0.01% of the IvG dose). IgG2X was BLQ in all fetal plasma samples. These data indicate that male-mediated mAb drug transfer via seminal fluid does not present a health risk to the female partner and is not bioavailable to the developing conceptus.

1. Introduction

There are limited experimental data assessing the potential for male-mediated developmental toxicity via drug transfer in seminal fluid, especially for large molecule biotherapeutics. Human risk assessments for this potential route of exposure have therefore been derived from conservative assumptions and extrapolation from small molecule drugs and/or endogenous moieties e.g. naturally occurring IgG. These conservative assumptions have consistently indicated that the risk of drug exposure via semen to achieve meaningful pharmacological levels in a pregnant woman or in the conceptus is negligible [1,2].

Circulating concentrations of small molecule drugs in pregnant women or the conceptus are projected to be at least 3 orders of magnitude lower than blood concentrations in the man whose semen is the alleged vehicle for drug transport, even for those rare drugs that bioaccumulate in seminal fluid to levels up to 11-fold higher than seen in blood [1]. For monoclonal antibody therapeutics (mAbs), substantially lower seminal fluid concentrations (~1% compared to serum levels; based on endogenous IgG data) [3,4] and their molecular weight, lipophilicity, and ionization characteristics in the vagina, indicate that prospective systemic exposure in the female would be even lower than described for small molecules [2]. Additionally, mAbs do not effectively cross the human placenta during organogenesis [5,6]. The placental transfer kinetics of IgG2X (the fully human IgG2 mAb used in this study) were recently characterized in cynomolgus monkeys and, consistent with the above, there was only minimal placental transfer of IgG2X during organogenesis. Embryonic systemic exposure to IgG2X was ≤0.5% of that seen in maternal plasma at this critical stage of development [7]. These various barriers to embryo–fetal exposure suggest that following administration to a male subject, mAbs would likely be non-bioavailable via seminal delivery to the developing conceptus.

The purpose of this study was to provide experimental data to assess the assumptions underpinning these conclusions. Specifically, this study focused on the extent of maternal and fetal exposure following repeated intravaginal (IvG) administrations of a fully human IgG2 mAb (IgG2X) throughout pregnancy in the cynomolgus monkey. IgG2X bound with high affinity to its human receptor target but did not cross-react effectively with

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the orthologous receptor in the cynomolgus monkey. This species difference enabled a direct assessment of potential Fc-mediated vaginal uptake to maternal circulation (and subsequent placental transfer to the conceptus) without the possible confounding interference of pharmacological target binding or target-mediated IgG2X clearance.

Potential routes of maternal/fetal exposure from male-mediated transfer have been elegantly reviewed by Klemmt and Scialli [1] (more detail provided in Section 4) and fall into two general categories – absorption from the vagina into the maternal circulation followed by placental transfer or direct transfer to the uterine cavity through the cervical canal. Based on their physicochemical properties, the latter seems biologically implausible for mAbs but the potential transfer via either route was investigated in this present study.

From confirmation of pregnancy on gestation day (gd) 21, IgG2X was administered IvG once every 2 weeks (biweekly; Q2W) to gd133. The objective here was to examine whether IgG2X maternal exposure potential via the IvG route changed during the course of pregnancy. The IvG formulation was optimized to mimic key physical properties of seminal fluid [8] and ensure sufficient viscosity to maximize vaginal retention. Care was also taken to position the animals after dosing in a manner that helped ensure retention of the intravaginal dose (see Section 2 for details).

Comprehensive pharmacokinetic (PK) analysis of maternal plasma samples was completed following the first dose on gd21 and before the second dose on gd35, to help ensure the optimal maternal blood sampling protocol was employed for the remainder of the study. Similar steps were taken after dosing on gd77 and prior to dosing on gd91 to detect any changes in the PK profile and further adjust maternal sampling adjusted as appropriate. At 72 h after the final dose on gd133, C-sections were performed and fetal blood samples were collected to determine the level of fetal IgG2X exposure.

2. Materials and methods

2.1. Materials

The fully human IgG2 monoclonal antibody, IgG2X (molecular weight, 145 kDa) used in these studies was formulated at an antibody concentration of 150 mg/mL in 10 mM sodium acetate, 9.0% (w/v) sucrose, 0.006% (w/v) polysorbate 20, pH 5.2. The formulation was selected based on the chemical and physical stability of mAbs at the selected pH and buffer composition, and was isotonic to avoid tissue irritation.

To investigate the optimal formulation for IvG dosing, the 150 mg/mL IgG2X drug substance was diluted to either 100 mg/mL or 120 mg/mL using the placebo buffer 2% hydroxypropyl methylcellulose (HPMC) in buffer consisting of 10 mM sodium acetate, 9.0% (w/v) sucrose, 0.006% (w/v) polysorbate 20, pH 5.2 (A52SuT). The drug substance was diluted using the 2% HPMC in the A52SuT buffer to increase the viscosity of the formulation in order to mimic the viscosity of seminal fluid [8] and ensure sufficient retention after IvG administration. The viscosity of the 100 mg/mL IgG2X formulation after dilution was 44 cP at 25 °C and 26 cP at 37 °C. The viscosity of the 120 mg/mL IgG2X formulation after dilution was 26 cP at 25 °C and 9 cP at 37 °C. The viscosity of the 120 mg/mL formulation, being only 9 cP at body temperature (37 °C), would not have been sufficiently high to ensure that the formulation would be retained after intravaginal administration compared to the 100 mg/mL formulation, hence the 100 mg/mL formulation was selected for dosing.

To assess IgG2X stability, an SEC-HPLC stability study of the 100 mg/mL IgG2X formulation diluted with 2% HPMC in A52SuT was performed. Stability samples were analyzed at $T = 0$, 3, 5, 7 and 11 days of storage. All samples, including the reference standard, were diluted to 1 mg/mL using mobile phase (100 mM sodium phosphate, 150 mM NaCl, pH 6.8). After 11 days of storage at 2–8 °C, there was minor growth in clipped species and the relative percent of the monomer remained above 98% purity. IgG2X was stable in the formulation, and no significant changes (<3%) in the SEC profiles were observed for 11 days. Based on these data, all the dosing formulations were made freshly at the testing facility and used within one week.

IgG2X bound to its human receptor (receptor X) target with a dissociation equilibrium constant (K_d) of 0.18 nM. In a functional assay, IgG2X inhibited ligand-induced receptor X activation on human B cells with a drug concentration producing 50% of maximum inhibition (IC_{50}) of approximately 160 pM. IgG2X did not cross-react effectively with the orthologous receptor in the cynomolgus monkey. For quantitation of IgG2X by immunoassay, a human receptor X:human Fc fusion protein used for the capture of IgG2X, as well as a human receptor X:human Fc fusion protein conjugated to Sulfo-TAG™ [for electrochemiluminescence immunoassay also referred to as MSD (Meso-Scale Discovery; Rockville, MD) assay] used for the detection of IgG2X were generated at Amgen Inc.

Female cynomolgus monkey K_2EDTA plasma was used as matched matrix for generating immunoassay standard curves and quality control (QC) samples.

2.2. Animals

Naïve, pregnant female cynomolgus monkeys (*Macaca fascicularis*, Chinese sourced), 4–12 years old and 3.1–4.9 kg (mean 3.7 kg) in weight at the time of dosing, were cared for in accordance to the *Guide for the Care and Use of Laboratory Animals, 8th Edition*, 2011 [9]. Animals were individually housed in species-specific housing at an indoor Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) international-accredited facility and socialized by providing grooming bar access to one another throughout the study. All research protocols were approved by the Institutional Animal Care and Use Committee.

Animals were fed a certified pelleted primate diet (PMI #5048, Richmond, IN) daily in amounts appropriate for the age and size of the animals, and had ad libitum access to municipal tap water (Reno, NV) processed through a reverse osmosis filter and UV light treatment, via automatic watering device. Animals were maintained on a 12:12 h light:dark cycle in rooms at 64–84 °F (17.8–28.9 °C) and 30–70% humidity, and had access to enrichment opportunities (device, food treat, and/or socialization). All animals were negative for simian retrovirus and tuberculosis.

2.3. Study design

After confirmation of pregnancy by ultrasound, 5 females were dosed IvG Q2W for a total of 9 doses of IgG2X (i.e. on gd21, 35, 49, 63, 77, 91, 105, 119, and 133).

For dose administration, each female was anesthetized with a long-lasting injectable anesthetic (ketamine/dexmedetomidine hydrochloride) and placed on a flat surface, laying face down with hips elevated upward (sternal recumbency), supported on a rolled towel. A disposable 1 mL prefilled sterile syringe was inserted into the vagina and advanced to reach as near the fornix as possible, then the IvG dose was administered over approximately 1 min. After dose administration, the syringe was removed and discarded. Each female was maintained under anesthesia in sternal recumbency for 30 min on a forced warm air blanket. Anesthesia was then reversed with atipamezole. Each female was observed for vaginal