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Farletuzumab, a monoclonal antibody directed against folate receptor alpha, shows no evidence of teratogenicity in cynomolgus monkeys

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ABSTRACT

Farletuzumab is a humanized monoclonal antibody targeting human folate receptor alpha, which is being developed as an anti-cancer drug. A non-human primate reproductive study was conducted to evaluate whether it could cause any embryonic or fetal abnormalities. Farletuzumab was administered intravenously to pregnant cynomolgus monkeys (n = 16/group) at doses of 0 or 67.5 mg/kg once weekly during gestation day (GD) 20 through 97. C-section was performed on GD100 ± 2, and fetuses were evaluated for morphologic (external, visceral and skeletal) effects. No farletuzumab-related changes were observed in maternal animals or fetuses, which are supported by the fact that farletuzumab has no effects on cellular uptake of folate. These data support the potential use of farletuzumab for oncologic indications during pregnancy.

1. Introduction

Folate is an essential nutrient needed for cells to generate DNA, RNA, and metabolic amino acids that are required for their proliferation and division. Since rapidly proliferating tissues require DNA synthesis the most, it is obvious that folate-dependent reactions are essential for fetal growth and development and that folate requirements increase during pregnancy 5- to 10-fold [1]. Animal studies demonstrated that folate deficiency causes intrauterine death, growth retardation, and various congenital malformations including cleft palate, skull defects, fore- and hindlimb dysplasias, and congenital heart defects [2,3]. The fact that folic acid supplementation in the periconceptional period decreases the risk of neural tube defects in humans suggests a causative role of folate deficiency in the etiology of these defects [4]. Several drugs have teratogenic effects through disturbing folate metabolism [5,6]. Methotrexate, sulfasalazine, triamterene, and trimethoprim are suggested to cause teratogenicity by inhibiting dihydrofolate reductase, which converts folic acid to the naturally bioactive form tetrahydrofolate. Other folate-disturbing teratogenic drugs include valproic acid, carbamazepine, and phenytoin, which are known to impair folate absorption or increase folate degradation.

The importance of folate receptors (FR) in embryogenesis has also been suggested. Inactivation of both alleles encoding the mouse homologue of the human folate receptor alpha (FRα) gene was uniformly fatal in embryos with neural tube defects [7,8]. Administration of an antiserum to FRs in pregnant rats resulted in the resorption of embryos or multiple developmental abnormalities in embryos: e.g., persistence of the curvature of the spine, hydrocephalus, abnormal cardiac and palatal morphogenesis [9], or severe learning deficits and cognitive impairment [10]. Rothenberg et al., first reported that serum from women who previously had a pregnancy complicated by neural tube defects contained autoantibodies that bound to the FRα and blocked the cellular uptake of folate *in vitro* [11]. Thereafter, many researchers have also suggested that high maternal antibody levels to FRα are regarded as a risk factor for congenital defects [12–15]. These findings prompted us to assess whether farletuzumab, an investigational antibody drug candidate to FRα, could cause any measurable and observable clinical changes during embryonic and fetal development.

Farletuzumab is a humanized monoclonal antibody of immunoglobulin G1κ targeting human FRα. As FRα is highly expressed in a variety of cancers but largely absent from normal tissue, farletuzumab is being developed for treatment of FRα-expressing cancers, including ovarian and non-small cell lung cancer [16–18]. Farletuzumab exerts its antitumor effect by induction of both antibody-dependent cell cytotoxicity and complement-dependent cytotoxicity in FRα-expressing tumors. Unlike the reported FR-autoantibodies, farletuzumab does not block FRα binding of folates nor affect folate delivery via FRα-mediated transport [19]. Cynomolgus monkeys were identified as a relevant

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species for the present study, since a high degree of homology (97%) exists in the overall amino acid sequences between cynomolgus monkey and human FRα, and the FRα epitope that is located between amino acids 45 and 57 of the FRα protein is identical between the two species [20]. Importantly, farletuzumab can bind to the cynomolgus monkey homolog of FRα with similar affinity as for human FRα. Herein, we report the effects of farletuzumab on embryo-fetal development in cynomolgus monkeys.

2. Materials and methods

2.1. Animals

The cynomolgus monkey was chosen for this study as it is the only pharmacologically relevant species. Species relevance was established by immunohistochemistry (IHC) using normal human and cynomolgus monkey matched tissues (see Supplemental Materials and Methods). The IHC results demonstrated a good correlation of pattern, as well as intensity of farletuzumab binding to cellular elements within known FRα-positive tissues, including kidney cortex (tubular epithelium), fallopian tubes, and ductal epithelium of pancreas (Supplemental Table 1). Conversely, no farletuzumab binding was found in tissues of either species known to be FRα-negative, including large intestine, small intestine, heart, sweat glands of the skin, striated muscle, bladder, and endometrium (Supplemental Table 1).

Female Chinese cynomolgus monkeys (Macaca fascicularis) were supplied from Charles River Laboratories. The females were sexually mature (4 to 8.2 years of age) and weighed between 2.7 and 4.7 kg. Animals were housed in the Testing Facility (Charles River Laboratories, Reno, NV) breeding colony during menstrual cycle checks, breeding, and pregnancy verification. The adult female was paired with a breeder male for 3 days, based on the anticipated timing of ovulation. The middle date of mating was considered GD0. While the adult female was in the breeding colony, pregnancy status was determined by ultrasound, once on GD18.

Animals were housed in stainless steel cages equipped with a stainless steel mesh floor and an automatic watering valve. Primary enclosures were as specified in the USDA Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and as described in the Guide for the Care and Use of Laboratory Animals. Temperatures of 64 °F to 84 °F (18 °C–29 °C), with a relative humidity of 30%–70%, were maintained. Ten or greater air changes per hour with 100% fresh air (no air recirculation) and a 12-hour light/12-hour dark cycle were maintained in the animal room. Purina Certified Primate Diet No. 5048 was provided daily, in amounts appropriate for the size and age of the animals. Females received between 20 and 40 biscuits per day during gestation. Processed water was available ad libitum to each animal via an automatic watering device.

This study was conducted in compliance with Good Laboratory Practice in a facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. All experimental procedures were pre-approved by the animal ethics committee of Charles River Laboratories.

2.2. Experimental protocol

The primary concern for farletuzumab based on mode of action was teratogenicity due to potential effects on folate levels and known effects of folate inhibitors based on literature. Also farletuzumab is being developed within the scope of International Conference on Harmonisation S9 guideline [21]. Therefore, C-section based embryo-fetal development toxicity study was sufficient to address this concern, rather than conducting an enhanced pre- and postnatal development study. Sixteen pregnant females each were assigned to the control (Animal Nos. 1501–1516) or farletuzumab-treatment group (Animal Nos. 2501–2516). The control article (phosphate buffered saline, pH 7.2/0.01% Tween 80 [w/v]) or farletuzumab 67.5 mg/kg were administered to animals via intravenous infusion (2.25 mL/min) by a suitable peripheral vein using a calibrated infusion pump. They were dosed initially on GD20 and once every 7 days through GD97 (12 doses). The dose of 67.5 mg/kg of farletuzumab was selected in anticipation of providing a significant margin of exposure over the highest dose expected to be administered to humans. The dosing period was intended to cover the period of major organogenesis (GD20 to 50) [22]. The start of dosing (GD20) was selected because this is the earliest practical time point at which pregnancy can be detected by ultrasound.

All animals were checked for mortality and clinical signs throughout the experimental period. Body weight was measured on the day of study enrollment GD20, GD25, and weekly thereafter. Ultrasound monitoring was conducted for pregnancy confirmation and embryo-fetal measurement. For a follow-up confirmation of pregnancy, serum levels of monkey chorionic gonadotropin (mCG) were measured only for animals with negative ultrasound results. All pregnant adult females were C-sectioned on GD100 ± 2. During C-section, amniotic fluid was collected for analyses of farletuzumab concentrations. Blood was collected from the fetal umbilicus for toxicokinetics (TK) and anti-farletuzumab antibody analyses. After fetal blood sample collection, each fetus was terminated under deep anesthesia induced with ketamine, pentobarbital sodium and phenytoin sodium solution. C-sectioned females were released to colony immediately after C-section.

2.3. Ultrasound monitoring for pregnancy confirmation and embryo-fetal measurement

Ultrasounds were performed to confirm pregnancy on GD18. During gestation, routine ultrasounds were performed approximately once every 2 weeks beginning on GD32 ± 1 day. Embryo/fetal viability was assessed by measuring the embryo/fetal heart rate. General condition of the pregnancy was assessed by examining the amniotic fluid volume and position of fetal presentation. In order to assess developmental landmarks, detailed ultrasounds were performed monthly, on 3 occasions: (GD45-47, 73–75, and 100 ± 2 [i.e., day of C-section]), on sedated adult females. Depending on practical considerations determined by age and size of the fetus or positioning of the fetus, developmental landmarks included, but were not necessarily limited to: greatest length (GL) of the embryo/fetus, biparietal diameter, occipitofrontal diameter, head circumference, and measurements of femur length (obtainable after GD50). GL, obtainable up to GD80, was determined by obtaining a sagittal scan through the midline and obtaining a maximum length from the top of the cranium to the base of the tail (crown/rump length).

2.4. Determination of mCG

Blood was collected by venipuncture on the first day of dosing (prior to dosing on GD20-GD22). The serum samples were stored at -60 °C until analysis. Two control females appeared to be not pregnant at ultrasound on GD33, and therefore in order to confirm pregnancy, the samples were analyzed for serum mCG by radioimmunoassay at Oregon National Primate Research Center.

2.5. Fetal evaluation

All fetuses were identified by Animal Nos. 1001–1007 for control males, 1101–1107 for control females, 2001–2010 for farletuzumab males, and 2101–2106 for farletuzumab females. Fetuses were evaluated for teratogenic endpoints, including external, visceral, and detailed cardiac and placenta/umbilical cord evaluations, body weight, organ weight, and morphometric measurement. Fetal external evaluation included morphometric measurement (crown/rump length, chest circumference, biparietal diameter, occipitofrontal diameter, horizontal head circumference, anogenital distance, femur length, and foot length). Fetal body weight and organ weight for brain, adrenal gland, kidney, liver, spleen, thymus, and placenta were collected.