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Characteristics of blood chemistry, hematology, and lymphocyte subsets in pregnant rhesus monkeys

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[ABSTRACT] The present study was designed to characterize the blood chemistry, hematology, and lymphocyte subsets in pregnant rhesus monkeys and provide baseline parameters for future studies of reproductive and developmental toxicity and developmental immunotoxicity. Harem-mating was used in 96 female and 16 male rhesus monkeys. Pregnancy was confirmed on gestation day (GD)18 by ultrasound. The blood samples of rhesus monkeys were collected at various times (20 days before pregnancy and GD20, 100 and 150). The analyses of blood chemistry, hematology, and lymphocyte subsets were performed. Copmpared with 20 days before pregnancy, Significant decreases ($P < 0.05$) were observed in HCT and RBC on GD20, GD150 and in HGB on GD150, Significant increases in NEUT and decreases in LYMPH on GD20 were observed. Significant decreases in ALB from GD20 to GD150 were observed, significant decreases in TP was observed on GD100. Significant increases in mean GLU were observed on GD20 and GD150 during pregnancy. Significant decreases ($P < 0.05$) in $CD20^+$ subsets on GD100, GD150 and $CD4^+/CD8^+$ ratio on GD150 were observed, The significant changes of MCV, MCHC , RDW-SD, MCV, MONO, ALT, AST, GLB, ALP, TBIL, DBIL, IBIL, GGT, CR-S, URIC, TC, TG and CK were observed during the pregnant period, but no biologic change were observed, There were no significant changes in MCH, RDW-CV, MPV, BUN, $CD3^+$, $CD4^+$ and $CD8^+$ during pregnancy. These data provide a database for preclinical study in rhesus monkeys. Physiological anemia, hyperglycemia, and immune suppression may occur in pregnant rhesus monkey which is similar to that found in human, and it is essential to distinguish the physiological changes from the pharmacological effects in reproductive and developmental toxicity and developmental immunotoxicity studies of pharmaceuticals.

[KEY WORDS] Pregnant rhesus monkeys; Hematology; Blood chemistry; Lymphocyte subsets

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Introduction

With the increasing discovery and development of pharmaceuticals and biopharmaceuticals, studies on the reproductive and developmental toxicity (DART) and developmental immunotoxicity (DIT) of test agents have drawn more and more attention. Although the rodents (mice and rats) are often used in the most non-clinical DART and DIT studies, the nonhuman primates (NHPs) are needed for studies of biopharmaceuticals [1-3]. The rationale behind this is that biopharmaceuticals often have high species specificity and that, compared with humans, rodents have significant different cross reactions to these agents and/or have higher immunogenicity, thus the testing results in rodents cannot accurately predict the potential toxicity in humans. In NHP studies, Rhesus monkeys are often used; they belong to the macaca mulatta family. Compared with rodents and rabbits, they have higher similarities with humans in phylogeny, morphophysiology, histological structure, immunology, physiological function, and metabolism; for example, female rhesus monkeys have regular menstrual cycles similar to humans and they have a similar placental transfer function with humans to monoclonal antibody drugs. In addition, as a large animal, they can afford more blood samples for laboratory tests and pharmacological studies [4-6].

In NHP DART studies, the drug dosing period for embryo-fetal development (EFD) toxicity testing is from gestation

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day (GD) 20 through GD50 (mainly the period of organogenesis), and the fetus are evaluated by caesarean section on GD100; the dosing period of peri- and post-natal development (PPND) toxicity testing is often from GD20 through the end of pregnancy or after parturition [7]. In addition, NHP DIT studies of biopharmaceuticals are often combined with EFD studies or PPND studies [8-10]. Therefore, the analysis of blood hematology, chemistry, and lymphocyte subsets from organogenesis to parturition is an important indicator for the evaluation of the drug's general toxicity and immunotoxicity. Several studies have reported the reference values for blood hematology, biochemical and lymphocyte subsets in non-pregnant rhesus monkeys as well as factors that influence these values, among which pregnancy is one of the main factors [11-15]. Considerable differences in blood hematology, chemistry, and immunological parameters have been reported between the non-pregnant and pregnant monkeys [16]. Accordingly, in the toxicity testing of pregnant rhesus monkeys, the reference values of non-pregnant monkeys may affect the interpretation of test results of given drugs. However, there are few reports on these reference values for pregnant rhesus monkeys. For example, Ibanez-Contreras *et al* [17] have only reported the changes in blood parameters of pregnant rhesus monkeys in the first 6−10 weeks of pregnancy; its implication in reproductive and developmental toxicity tests may be limited. The objectives of the present study were to analyze the changes in maternal blood hematology, chemistry, and lymphocyte subsets from the period of organogenesis to delivery and to provide a database to support the use of rhesus monkeys in non-clinical DART and DIT studies.

Materials and Methods

Animals

A total of 96 female non-pregnant rhesus monkeys and 16 male rhesus monkeys (aged 4−7 years and weighing 5.0−8.5 kg) were obtained from the National Experimental Rhesus Monkey Resources Base, Ya'an, Sichuan, China. Animal quarantine procedures included physical examinations, intradermal skin test for mycobacterium tuberculosis, fecal examination for parasites, bacterial culture for salmonella andshigella, and serology for herpes B virus. The results were all negative.

Mating and pregnancy confirmation

The Harem-mating method was used in the present study. Among the 96 female rhesus monkeys, every six female monkeys were allowed to cohabit with one male for 7 days. When copulation was confirmed visually, the median day of the mating period (day 4 of mating) was designated as Day 0 of gestation (GD0). On GD18 the pregnancy was confirmed by ultrasonography (DP 50Vet, Mindray Co., Ltd., Shenzhen, China). The animals were anesthetized by an intramuscular injection of 5% ketamine hydrochloride (5–10 $mg \cdot kg^{-1}$, Bioniche Teoranta Inverin, Co., Galway, Ireland). The pregnant monkeys were raised in stainless steel monkey cages (2 m long, 0.9 m wide, and 2 m high, 1 animal per cage) in a feeding room with the temperature of 18 to 26 ºC, humidity of 50% to 70%, and 12 h lighting. The monkeys were provided daily with 200 g primate chow (18% protein, 69% carbohydrates, 10% water, and 3% fat) and 50 g apples. The monkeys were maintained in conformity with the requirements of "the National Institutes of Health Guide for the Care and Use of Laboratory Animal'' of the United States, and all the experimental protocols were reviewed and approved by the Animal Welfare and Use Committee, Sichuan Primed Bio-tech Shines Co., Ltd., Chengdu, China).

Blood sample collection and analyses

After 12 h fasting, the blood samples were collected via cephalic veins from each animal, at 8: 00 to 9: 00 am on GD20, GD100, and GD150. Each sample was divided into two aliquots: one for hematology and another for analysis of lymphocyte subsets. Hematology tests were carried out with Hematology (XT-2000i, Sysmex, Tokoyo, Japan); the parameters included in the present study were as follows: white blood cell count (WBC, 10^{12}/L), red blood cell count (RBC, 10^{12}/L), hemoglobin (HGB, $g \cdot L^{-1}$), hematocrit (HCT, %), mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, $g \cdot L^{-1}$), platelet count (PLT, 10^9/L), red blood cell distribution width-standard deviation (RDW-SD, Fl), red blood cell distribution width coefficient of variation (RDW-CV, %), Platelet volume distribution width (PDW, Fl), mean platelet volume (MPV, Fl), neutrophile count (NEUT, 10^9/L), lymphocyte count (LYMPH, 10^9/L), and monocyte count (MONO, 10^9/L). For the analysis of lymphocyte subsets, the cells were acquired with a three-color flow cytometry (Becton Dickinson, San Diego, CA, USA), and analyzed by Cell Quest software (Becton Dickinson, San Diego, CA, USA), The serum samples were used for chemistry test by automated biochemistry analyzer Synchron CX4 PRO (Beckman Coulter, Brea, California, USA). The parameters in blood chemistry tests included the following: alanine aminotransferase (ALT, $IU \cdot L^{-1}$), aspartate aminotransferase (AST, $IU \cdot L^{-1}$), total protein (TP, $g \cdot L^{-1}$) , albumin (ALB, $g \cdot L^{-1}$) , globulin(GLB, $g \cdot L^{-1}$), alkaline phosphatase (ALP, $IU \cdot L^{-1}$), total bilirubin (TBIL, $\mu mol \cdot L^{-1}$), direct bilirubin (DBIL, $\mu mol \cdot L^{-1}$), indirect bilirubin (IBIL, $\mu mol \cdot L^{-1}$), gamma glutamyl transferase (GGT, $IU \cdot L^{-1}$), creatinine (CR-S, $\mu mol \cdot L^{-1}$), urea nitrogen (BUN, $mmol \cdot L^{-1}$), Glucose (GLU, $mmol \cdot L^{-1}$), uric acid (URIC, $\mu mol \cdot L^{-1}$), creatine kinase (CK, $IU \cdot L^{-1}$), cholesterol (TC, $mmol \cdot L^{-1}$), and triglyceride (TG, $mmol \cdot L^{-1}$) .

Statistical analysis

All data are expressed as mean ± SD. Significances of the differences in the test values between non-pregnancy (GD-20) and pregnancy (at different days after pregnancy) were determined using one-way ANOVA. All statistical analyses were performed using IBM SPSS version 19 (SPSS Inc., Chicago, IL, USA).

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