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Embryo–fetal exposure and developmental outcome of thalidomide following oral and intravaginal administration to pregnant rabbits

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ABSTRACT

Studies in pregnant rabbits were conducted to evaluate if there are any differences in the uptake of thalidomide into the intrauterine compartment and developmental toxicity risk following oral and intravaginal administration. Thalidomide concentrations in maternal plasma, yolk sac cavity (YSC) fluid and embryo following intravaginal administration were 2- to 7-fold lower than their respective levels after oral administration. Ratios of thalidomide concentration in YSC fluid to maternal plasma were similar between these two routes, indicating no difference in uptake into the intrauterine compartment. A rabbit embryo–fetal development study using oral and intravaginal thalidomide administration at 2 mg/kg/day (a dose >10,000-fold higher than the expected amount of thalidomide in human semen) did not result in any developmental abnormalities. These data demonstrated no preferential transfer mechanism of thalidomide from vagina to conceptus, and no additional embryo–fetal developmental toxicity risks with thalidomide exposure via the vaginal route.

1. Introduction

Thalidomide has a long and well-known regulatory history; it was approved in the late 1950s for the treatment of nausea associated with morning sickness, but its use was banned in the early 1960s after approximately 10,000 babies were born with phocomelia [1]. Thalidomide is currently an approved drug for treatment of multiple myeloma and erythema nodosum leprosum. Due to its teratogenic concerns, thalidomide is only available through a restricted distribution program [2]. In a male rabbit study using orally administered [^{14}C]-thalidomide [3], radioactivity was detected in rabbit seminal fluid, and its level generally correlated to the level of radioactivity in the plasma samples. Ratio of thalidomide concentration in semen ejaculate to concentration in plasma at 6 h post-dose ranged from 1.3 to 1.4. Thalidomide was also found in semen of two human patients after oral administration [4]. Concentrations were variable, but there was an apparent correlation between plasma and semen levels. Based on this information, there is a guidance in the prescribing information [2] for the male patients to use a condom during any sexual contact with females of reproductive potential while taking thalidomide and for up to 28 days after discontinuing thalidomide, even if they have undergone a successful vasectomy, and male patients taking thalidomide must not donate sperm.

Transport to semen has been demonstrated for numerous chemicals and drugs [5], and a review of this topic was published by Klemmt and Scialli [6]. As in the case of thalidomide, there has been safety concerns about the potential embryo–fetal harm following seminal exposure of drugs with teratogenic potential. Based on the weight of ejaculate and the concentration of drug present in semen, the amount of drug available to be delivered to the conceptus is generally very low, unless there is a preferential route of drug transport that results in much higher drug concentrations in the uterine compartment. There are three proposed mechanisms for exposure of the conceptus to drugs via semen [6]:

1. Absorption of drugs from the vagina to the maternal circulation.
2. Delivery of drugs to the egg and conceptus via binding to the sperm cell.
3. Direct transport of drugs from the vagina to the uterine cavity.

The authors cited examples where the mechanisms of vaginal absorption of drugs into the maternal circulation and binding of drugs to sperm cells were demonstrated. However, there is not a clear understanding on the mechanism of direct delivery of seminal chemicals into the uterine cavity of human. This direct delivery route, if it exists, should result in a higher uterine drug concentration, compared to plasma, when dosing is given intravaginally.

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A countercurrent mechanism or uterine first pass effect has been proposed [7–9] to explain the observation that the intravaginal administration of progesterone results in higher ratios of endometrial to serum progesterone concentration than does intramuscular progesterone administration to postmenopausal women. Countercurrent transport is a physiological exchange mechanism between fluids flowing in opposite directions, in this case, from the vaginal vein to the uterine artery. However, this uterine first pass effect has not been demonstrated with drugs other than progesterone given intravaginally, or in premenopausal, reproductive-aged or pregnant women [6]. The authors also noted that the epithelium of the menopausal vagina is thinner than vaginal epithelium during the reproductive years.

The purposes of the current studies are to investigate if there is any difference in uptake of thalidomide into the intrauterine compartment following oral and intravaginal administration to pregnant rabbits (toxicokinetic study), and if there is an increased developmental toxicity risk when thalidomide is given intravaginally, compared to oral administration (embryo–fetal development study). Rabbit was selected as a model for this investigation because it is well known as a sensitive species to thalidomide-induced embryopathy, and it is a species that intravaginal dose administration is feasible.

2. Materials and methods

2.1. Testing facilities and test sites

The in-life and formulation analysis portions of the studies were conducted at MPI Research, Inc. (Mattawan, MI). The bioanalysis and toxicokinetic evaluation of thalidomide in rabbit plasma, yolk sac cavity (YSC) fluid and embryo samples from the toxicokinetic study were performed by Celgene Corporation (Summit, NJ), and XenoBiotic Laboratories, Inc. (Plainsboro, NJ) conducted these evaluations for the plasma samples from the rabbit embryo–fetal development (EFD) study.

2.2. Formulation of test and control articles

Vehicle for oral gavage administration was 1% carboxymethylcellulose (medium viscosity) in deionized water, pH 4.4 ± 0.1; this was stored refrigerated at 2–8 °C when not in use. Fresh control article for intravaginal administration, Carbopol 974P, NF (0.30%)/methylcellulose (4000 cps) (1.00%)/glycerin (5.00%), USP/methylparaben, NF (0.10%)/propylparaben, NF (0.05%)/purified water, USP (pH 4.4 ± 0.1) (93.55%), was prepared for use on study weekly and stored refrigerated at 2–8 °C when not in use.

Thalidomide batch 0848S087 was used in the toxicokinetic study and batch 574-574-09-003 was used in the EFD study. These batches had assayed chemical purities of >99%, and therefore, no adjustment was made for purity when preparing the formulations. Formulations of thalidomide for oral and intravaginal administration were prepared in their respective vehicles, and were stored refrigerated at 2–8 °C when not in use.

Dosing formulations prepared for the study were evaluated for concentration and homogeneity prior to dosing. Samples were collected using a positive displacement pipette or syringe, and placed into amber glass bottles or scintillation vials prior to analysis. The oral formulation samples were collected while stirring, and samples were collected from top, middle and bottom stratum of the formulations. For intravaginal formulations, homogeneity samples were collected from six evenly distributed areas of the container. All samples were assayed by using validated methods. In addition, both intravaginal and oral formulations were demonstrated to be stable under refrigerated (2–8 °C) conditions for at least 13 days at the concentrations used in the studies.

2.3. Standards and reagents

Reference standard for thalidomide (>99% chemical purity) was synthesized by Celgene Process Chemistry (Summit, NJ) and stable label thalidomide-d_4 (98% chemical purity), used as internal standard in the analytical assay, was purchased from Toronto Research Chemicals, Inc. (Ontario, Canada). Control New Zealand White rabbit plasma was purchased from Bioreclamation (Hicksville, NY). All other reagents and chemicals were obtained from commercial sources.

2.4. Animals and husbandry

Time-mated female New Zealand White Hra:(NZW)SPF rabbits used in the studies were received from Covance Research Products, Inc. (Greenfield, IN). Forty-four animals were used in the toxicokinetic study and 94 animals were used in the EFD study. These studies were designed to use the fewest number of animals possible, consistent with the objective of the study, the scientific needs and contemporary scientific standards, to allow sufficient group sizes for meaningful analysis of data. The animals were acclimated from the time of arrival on gestation day (GD) 0 until the time of dosing on GD 7. During the acclimation period, the animals were observed for general health and any signs of disease, and had body weight and food consumption measurements collected. During the acclimation period, the animals were fed approximately 50 g of basal diet (Lab Diet® Certified Rabbit Diet #5322, PMI Nutrition International, Inc.) on the day of arrival at the Test Facility, and approximately 170 g starting on the second day of acclimation, and continued throughout the study period. Water was supplied ad libitum to all animals via an automatic water system. The rabbits were individually housed in suspended, stainless steel, slatted floor cages. Fluorescent lighting was provided via an automatic timer for approximately 12 h per day. Temperature and humidity were monitored and recorded daily and maintained between approximately 61–72 °F and 30–70%, respectively.

2.5. Toxicokinetic study

2.5.1. In-life procedures

In the toxicokinetic study, thalidomide was administered daily via oral or intravaginal route to time-mated rabbits beginning on GD 7 and continuing through GD 11. Oral dose was administered by gavage. One group of two animals was administered the oral vehicle, and two groups of eight animals per group were administered the oral thalidomide formulations at dose levels of 20 or 180 mg/kg/day. These oral dose levels were selected on the basis of available embryo–fetal development data from previous rabbit studies [10]; classical thalidomide embryopathy was observed at 180 mg/kg/day but not at 20 mg/kg/day. One group of two animals was administered the intravaginal control article intravaginally, and three groups of eight animals per group were administered thalidomide intravaginally at dose levels of 2, 20 or 180 mg/kg/day. A lower intravaginal dose of 2 mg/kg/day was included as a better representation of drug exposure via semen than doses of 20 and 180 mg/kg/day, and was the lowest dose that could be used based on the assay sensitivity for thalidomide concentrations in plasma samples. The oral doses were given at a dose volume of 4 mL/kg/dose and the intravaginal doses were administered at a dose weight of 0.5 g/kg/dose. Individual doses were based on the body weights collected the day prior to each dose. Animals that were dosed intravaginally were checked visually for leakage of formulation from vagina post-dosing.