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The use of optical imaging to assess the potential for embryo-fetal exposure to an exogenous material after intravaginal administration

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ABSTRACT

A β-actin-luc transgenic mouse model was used to evaluate whether embryo-fetal exposure could occur after intravaginal administration of a compound. A bioluminescent substrate, D-luciferin, was delivered intravaginally to mimic compound exposure to the female reproductive track and the embryo-fetus. Bioluminescence was observed throughout the reproductive tract during diestrus, but not during estrus, 2–5 min after intravaginal D-luciferin administration to female β-actin-luc mice. Intravaginal administration of D-luciferin to wild-type females mated with male β-actin-luc mice indicated that the substrate reached the developing embryo-fetus, with bioluminescence corresponding to transgene expression in the embryo-fetus. D-Luciferin substrate rapidly reached the embryo-fetus regardless of the administration route (intravaginal, intraperitoneal, subcutaneous, or intravenous). Vaginal ligation appeared to block at least some direct exposure to the embryo-fetus, but did not prevent D-luciferin from eventually reaching the embryo-fetus. Additional work will be necessary to form the basis for a reliable assessment of the human risk for male-mediated teratogenicity.

1. Introduction

It has been well established that many drugs and chemicals have the potential to cause harm to a developing embryo-fetus. The types of effects can range from congenital malformations to spontaneous abortions and still births. The typical studies employed during drug development to investigate the ability of a compound to cause fetal malformations look at maternal exposures that occur during the gestation period, and thus these effects would be considered maternally-mediated. There are a number of drugs that have been shown to cause human birth defects, with the most notable being thalidomide. Exposure of pregnant women to the drug thalidomide in the late 1950s and early 1960s for the treatment of nausea resulted in severe birth defects in thousands of children [1].

Investigations have turned to the potential role for male-mediated effects as a potential cause of adverse outcomes in offspring. Studies have shown that drugs and chemicals can enter the seminal fluid and, in some circumstances, can achieve concentrations much greater than what is seen in blood [2]. Drugs present in the seminal fluid could enter the female reproductive tract during sexual intercourse and potentially interfere with embryo-fetal development. For example, cyclophosphamide, an anticancer and immunosuppressant drug, has been shown to enter the seminal fluid of rats [3]. Using [14^C]-cyclophosphamide administered intravenously to male rats, radiolabel was first found in seminal fluid within 10 min and was equilibrated with the plasma within 30 min. To assess the potential effects of paternal cyclophosphamide treatment on pregnancy outcome, male rats were administered a single dose of cyclophosphamide immediately prior to cohabitation with untreated female rats. There was a significant decrease in the number of implantations and live fetuses per pregnant female [3], demonstrating that cyclophosphamide can enter the seminal fluid, be transmitted to the female partner, and affect progeny outcome.

Few studies have investigated the potential mechanisms for exposure of the conceptus to drugs or chemicals in semen. Several mechanisms have been proposed (see review by Klemmt and Scialli [2]) and are briefly listed here: (1) access of drugs or chemicals to the maternal circulation after absorption from the vagina, (2) direct exposure of the conceptus following transport from the vagina to the uterus, and (3) delivery to the egg, and subsequent conceptus, of chemical bound to the sperm cell.

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Recently, regional health authorities have expressed some concern over paternally-mediated embryo-fetal harm following paternal exposure to drugs. This has led drug developers to require barrier protection (i.e., condoms) for men enrolled in clinical trials to safeguard their female partner in case of pregnancy; the issue being that the concentration of drugs in semen had not been routinely assessed by most companies. Since humans may continue to have sexual intercourse during pregnancy, there could be a potential for an embryo-fetus to be exposed to a higher localized concentration of a drug relative to maternal systemic exposure.

There is very limited information on the drug concentration in the embryo-fetal compartment and, in an effort to generate data on this issue, the International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI), Developmental and Reproductive Toxicology Technical Committee (DART TC) coordinated a series of experiments. In the study presented here, we used the β-actin-luc transgenic mouse and optical imaging to investigate the potential for a developing embryo-fetus to be exposed to a drug administered via the vagina. We administered a volume of the D-luciferin substrate into the vagina of the mice to simulate deposition of semen into the vagina during sexual intercourse, and then visualized its expression throughout the female reproductive tract and the embryo-fetal compartment. Since the expression of the luciferase gene is ubiquitously expressed in nearly all tissues of the transgenic mouse, including the reproductive tract, detection of light (from the interaction of the luciferase protein with the D-luciferin substrate) identified the location and confirmed the distribution of the D-luciferin substrate in the female reproductive tract and embryo-fetal compartment.

2. Materials and methods

2.1. Animals

All studies described in the paper were approved by Sanofi's IACUC under laboratory animal protocol BW-AUP-1011. FVB wt/NTac mice and FVB β-actin-luc transgenic mice were purchased from Taconic (Germantown, NY). The transgenic line carries a 14 kb fragment of the murine β-actin promoter, a chimeric intron, and a modified firefly luciferase cDNA reporter gene (Promega pGL3). The whole expression cassette was randomly integrated in the FVB mouse genome. The luciferase reporter is constitutively expressed in nearly all tissues, including male and female reproductive organs. β-actin-luc transgenic mice are hemizygous and were derived from FVB/NTac mice. This transgenic reporter line has been used to study the transplantation of various tissue and cell types [4]. Pregnant mice were obtained by mating wild-type females with hemizygous β-actin-luc transgenic males or by mating transgenic male and female mice. The day of mating confirmation was designated as Gestation Day 0 (GD0).

2.2. Mouse estrous cycle detection

The mouse estrous cycle lasts approximately 4 days, and consists of four stages: proestrus (13 h), estrus (15 h), metestrus (13 h), and diestrus (56 h) [5]. Vaginal cytology was used to determine the estrous cycle stages [6]: the stage of the estrous cycle was determined based on the proportions among three cell types (nucleated epithelial, cornified epithelial and leukocytes; see Byers et al. [6] for vaginal cytology details).

2.3. Anesthesia, shaving and intravaginal dosing

Mice were anesthetized by isoflurane inhalation for the duration of the imaging conducted on any given day. The fur on the dorsal and ventral trunk was shaved close to the tail with an electric clipper and an attached number 10 blade to optimize the imaging signal from the uterine horn and embryos-fetuses. A calibrated micropipette with disposable tips was used for intravaginal dosing of 25–50 μL of bioluminescence substrate (D-luciferin, 0.5 mg/mL for estrous cycle imaging and 25 mg/ml for transgenic embryo-fetal imaging) or fluorescence substrate (XenoLight RediJect D-luciferin Ultra [pre-formulated batch controlled D-luciferin mixed with fluorescent marker; PerkinElmer, Catalog number 760505]).

2.4. Bioluminescence imaging

By combining animal genetic engineering and molecular imaging techniques, many transgenic models carrying a bioluminescent reporter have been generated [7]. Bioluminescence, the enzymatic generation of visible light, has an exceptionally high signal-to noise ratio in mice and rats, thereby making it an extremely sensitive technique. Firefly luciferase is widely used in many models [8]. D-Luciferin potassium salt (MW = 318) is a small-molecule substrate that is oxidized in the presence of the enzyme luciferase and ATP/oxygen to produce oxyluciferin and energy in the form of light. D-Luciferin at doses up to 250 mg/kg, based on solubility and injection volume limitations, can be adminstered to animals through various routes with no evidence of toxicological or immunological effects [9]. A Xenogen IVIS 100 imaging system (PerkinElmer, Hopkinton, MA) was used for bioluminescence acquisition. A low substrate concentration (0.5 mg/mL) was used for estrous cycle imaging in the transgenic β-actin luc female mice since high concentrations (i.e., >5 mg/mL) resulted in the appearance of a large bright amorphous image due to bioluminescent signal also being generated by the surrounding abdominal tissue. However, a high concentration of substrate (i.e., 25 mg/mL) was used to increase image sensitivity for the transgenic embryo-fetal imaging studies since no interfering bioluminescent signal was generated from the wild-type adult female mouse tissue.

In vivo images were captured immediately after dosing once the anesthetized mice were placed in the imaging chamber (exposure 1–5 s every 1–2 min, up to 20 min, with additional imaging performed at several hours to days after dosing). To better visualize imaging substrate distribution in the mouse reproductive tract, both dorsal and ventral views were imaged. For pregnant mice, we limited imaging to three times (from pre-implantation to mid-gestation) since repeated administration of isoflurane is considered a significant physiological stressor for both the dam and offspring under the Sanofi IACUC policy on the humane use of animals in research.

2.5. Fluorescence imaging

Fluorescence imaging was used to demonstrate the validity of the bioluminescence imaging technique. We used a Xenogen IVIS Lumina imaging system for near infared florescent imaging and either IRDye 800 CW NHS Ester (MW = 1166, Invitrogen (now Life Technology, Grand Island, NY)) or XenoLight RediJect D-Luciferin Ultra (PerkinElmer, Hopkinton, MA) containing the fluorescence marker. For the IVIS Lumina system, we used an ICG filter set, excitation pass band 710–760 nm, and emission pass band 810–875 nm.

2.6. Vaginal ligation

The abdomen of the mouse was shaved using electric clippers with a number 10 blade attached. An incision was made longitudinally through the abdominal wall from just below the rib cage to the pubic bones. The incision was extended laterally on both sides of the midline to expose the uterus. Small forceps were used to separate the connective tissues and expose the vagina. Surgical suture was threaded around the vagina just ventral to the