Reproductive Toxicology 59 (2016) 1–7

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Assessment of cervical passage of vital dyes in pregnant, nonpregnant, and mated rats and mice

Kary E. Thompson[a,*], Stephanie L. Rayhon[a], Graham Bailey[b], Peter Delille[b], Mary Ellen McNerney[a]

[a] Department of Reproductive Toxicology, Drug Safety Evaluation, Research & Development, Bristol-Myers Squibb Company, New Brunswick, NJ 08903, USA
[b] Preclinical Development & Safety, Janssen Research & Development, 2340 Beerse, Belgium

ARTICLE INFO

Article history:
Received 31 July 2015
Received in revised form
23 September 2015
Accepted 26 October 2015
Available online 4 November 2015

Keywords:
Cervical passage
Risk assessment
Vital dyes
Intravaginal
Semen

ABSTRACT

Risk assessment for indirect exposure to small molecule pharmaceuticals in semen to the conceptus has traditionally been handled by calculations based on assumptions that any embryo–fetal exposure would be secondary to maternal absorption and redistribution. This study was designed to assess the potential for transcervical passage of drugs from semen. Reproductive tracts of rodents were examined following vaginal dosing with vital dyes during the estrous cycle, mating, and pregnancy. Toluidine Blue was not observed beyond the cervix after vaginal administration in pregnant rats; additionally, it did not pass the cervix in rats during any phase of estrous. In order to address the effects of semen, rats were dosed at receptivity and mated. Vital dyes were not visually evident in the uterus despite vaginal and sperm plug staining. This study provides evidence that direct transcervical passage is not a substantial route of direct embryo–fetal exposure for small molecule drugs in semen.

1. Introduction

Risk assessment and the supporting nonclinical studies for developmental toxicity are largely centered on maternal use of pharmaceuticals and potential sequelae for the conceptus [1]. However, for small molecule drugs, it is known that these compounds, when taken by men, distribute to the seminal compartment. Given that there are small molecule pharmaceutical agents designed for vaginal administration, which either act locally, or are absorbed by the vaginal epithelium and result in systemic exposure [2], it is expected that female sexual partners of men taking small molecule pharmaceuticals will be vaginally exposed to drugs in semen [3]. Since vaginal administration is a known means of small molecule absorption in women, accordingly, this would represent inadvertent systemic exposure to some extent to small molecule pharmaceuticals in women with no concomitant disease. In cases where female partners are pregnant, this concern for secondary exposure extends to the developing conceptus, as development represents a critical window of sensitivity.

When there is a concern for a particular drug (e.g., due to mechanism of action and/or lack of a developmental no-observable adverse effect level identified in an embryo–fetal development study), modeling the exposure to drugs in semen via vaginal absorption from the vaginal mucosa into maternal systemic circulation and subsequent redistribution to the conceptus is traditionally employed to assess for a potential risk using a series of worst-case scenario assumptions [3]. However, it is unknown whether other potential mechanisms could contribute to embryo–fetal exposure to drugs in semen and result in accumulation beyond that predicted by theoretical estimates. Direct trans-cervical passage by passive diffusion of small molecules delivered in semen has been hypothesized as a potential mechanism to result in locally-higher concentrations of drug within the embryo/fetal compartment, although there is an absence of empirical evidence directly evaluating this in vivo. As such, the potential contribution of trans-cervical passage to embryo–fetal exposure to drugs in semen is unknown.

In humans, seminal fluid remains in the vagina, blocked from cervical passage by mucus; additionally, sperm penetration through the cervical mucus is limited to the period of ovulation [4,5] when estrogens alter or "thin" the mucus consistency and permit access to the uterine compartment [6]. With an established pregnancy, a dense cervical mucus plug is maintained throughout gestation to serve as protection from pathogens and other molecules [7,8]. Based on this physiology, it has also been con-

* Corresponding author at: Bristol-Myers Squibb Company, 1 Squibb Drive, New Brunswick, NJ 08903, USA. Fax: +1 732 227 3218.
E-mail address: kary.thompson@bms.com (K.E. Thompson).

http://dx.doi.org/10.1016/j.reprotox.2015.10.018
0890-6238/

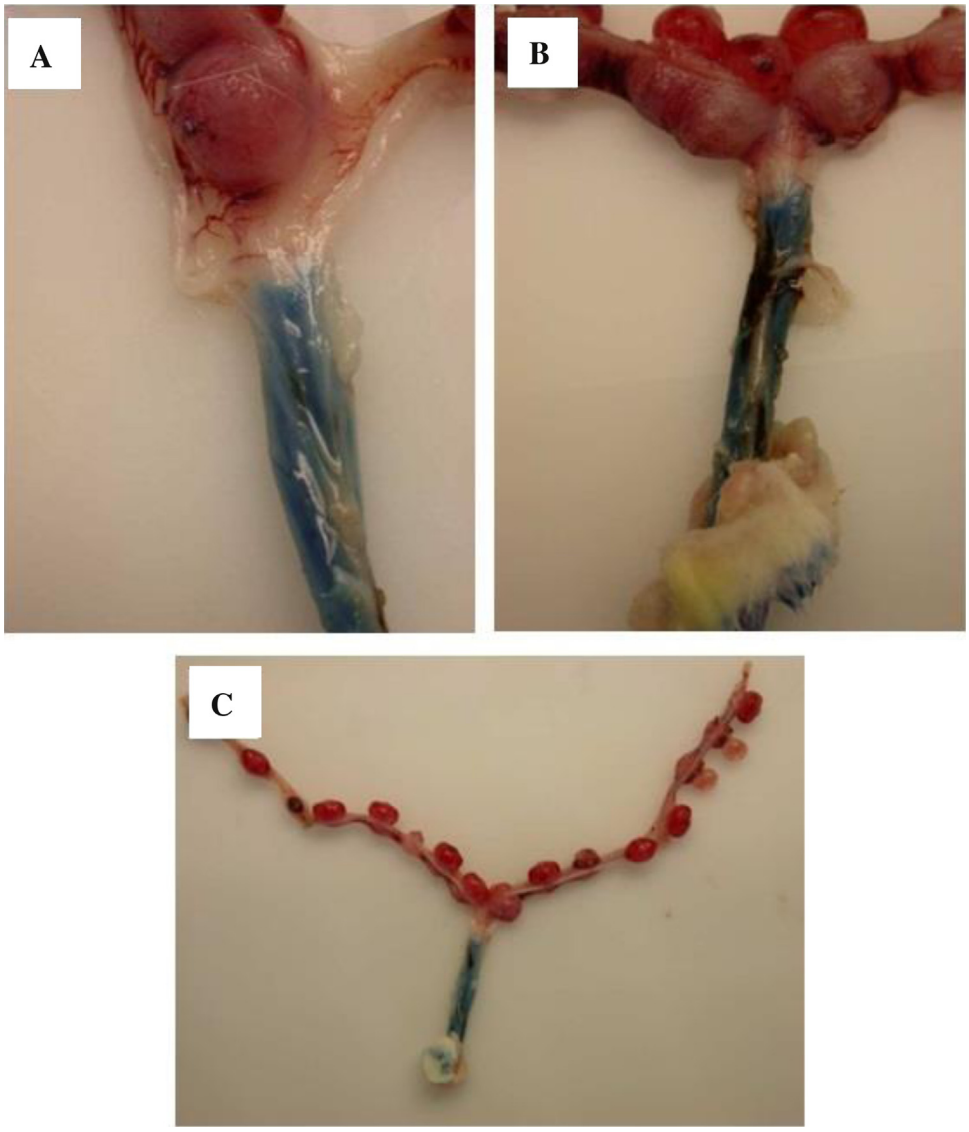

Fig. 1. Reproductive tract from Gestation Day 12 rats collected at timed intervals after intravaginal dosing with Toluidine Blue illustrating non-penetrance of the dye past the cervix into the uterus—A: 15 min postdose, B: 120 min postdose, C: 240 min postdose.

jectured that drugs in semen may adhere to spermatozoa and be delivered to the oocyte at the time of fertilization [3]. While the amount of drug that could be directly carried by a spermatozoa into the potential zygote is likely minimal, there is a lack of experimental data assessing this possibility.

The objective of this project was to generate experimental evidence in animals to assess whether the theoretical concern for trans-cervical passage of drugs in semen is an unmodeled route of exposure to the embryo/fetus. This study was specifically designed to visually assess for potential movement of small molecules across the cervix in rats and mice following vaginal administration of vital dyes of various molecular weights and properties during mid gestation. As rodents (unlike humans) will not mate during pregnancy, the ability of semen to move vital dyes into the uterus could not be assessed during gestation in these studies. Therefore, to explore the potential influence of seminal fluid and sperm to affect the trans-cervical movement of vital dyes, additional studies were conducted with cohabitated female rats during proestrus. This work was conducted as part of the Health and Environmental Sciences Institute (HESI) Developmental and Reproductive Toxicology (DART) Drugs in Semen Consortium [9].

2. Materials and methods

2.1. Dosing formulations

Toluidine Blue (CAS# 6586-04-5) was prepared as an aqueous 10% (w/v) solution in sterile water (Butler-Schein B111201-2). Sudan Black B (CAS# 4197-25-5), Brilliant Green (CAS# 633-03-4), Acid Blue 29 (CAS#5850-35-1), and Acid Blue 40 (CAS# 6424-85-7) were prepared at 10% w/w in water-based lubricating jelly (5% hydroxyethyl cellulose/15% glycerin in phosphate buffer, pH 7.1; KY Jelly, McNeill-PPC Inc. or Rite-Aid). In the absence of specific vaginal formulations for these dyes; these vehicles were considered acceptable "off-the-shelf" options for vaginal dosing based on animal welfare. All dyes were purchased from Sigma–Aldrich, and formulations were prepared once on the day of dose administration.

2.2. Animals

Virgin female mice [Crl:CD-1(ICR)BR], approximately 9–10 weeks old, 24–29 g and virgin female rats [Crl:CD(SD)],