Differentiation 91 (2016) 1–18

Contents lists available at ScienceDirect

Differentiation

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

Review article

Diethylstilbestrol-induced mouse hypospadias: "window of susceptibility"

Adriane Watkins Sinclair [a], Mei Cao [a], Laurence Baskin [a], Gerald R. Cunha [a,*]

[a] Department of Urology, University of California San Francisco, 400 Parnassus Avenue, Box A610, San Francisco, CA 94143, United States

ARTICLE INFO

Article history:
Received 14 November 2015
Accepted 7 January 2016
Available online 20 January 2016

Keywords:
Diethylstilbestrol
Estrogen
Mouse penile development
Urethra

ABSTRACT

This review presents published and novel results that define the programming window for diethylstilbestrol (DES)-induced abnormal development of the mouse penis. These data indicate that DES has its greatest effect during the period of most intense penile morphogenesis, namely postnatal days 0-15 (P0-P15). Pregnant mice and their neonatal pups were injected subcutaneously with 200ng/gbw DES every other day from embryonic day 12–18 (DES E12–E18), postnatal day 0–10 (DES P0–P10), embryonic day 12 to postnatal day 10 (DES E12–P10), postnatal day 5–15 (DES P5–P15), and postnatal day 10–20 (DES P10–P20). Aged-matched controls received sesame oil vehicle. After euthanasia at 10, 15, 20 and 60 days, penises were analyzed by gross morphology, histology and morphometry. Penises of all 5 groups of DES-treated mice were reduced in size, which was confirmed by morphometric analysis of internal penile structures. The most profound effects were seen in the DES E12–P10, DES P0–P10, and DES P5–P15 groups, thus defining a DES "programming window". For all parameters, DES treatment from P10 to P20 showed the most mild of effects. Adverse effects of DES on the MUMP cartilage and erectile bodies observed shortly after the last DES injection reverted to normality in the DES P5–P15, but not in the E12–P10 and P0–P10 groups, in which MUMP cartilage and erectile body malformations persisted into adulthood, again emphasizing a "window of susceptibility" in the early neonatal period.

Contents

Abbreviations: DES, Diethylstilbestrol; E, embryonic; P, postnatal; OPT, optical projection tomography

* Corresponding author.
E-mail address: gerald.cunha@ucsf.edu (G.R. Cunha).

http://dx.doi.org/10.1016/j.diff.2016.01.004
Join the International Society for Differentiation (www.isdifferentiation.org)
0301-4681/

1. Introduction

Hypospadias is the second most common urogenital anomaly in boys occurring in approximately 1:200–1:300 male births (Baskin, 2000), and the incidence of this congenital defect in the USA has doubled in recent times (Paulozzi et al., 1997; Paulozzi, 1999). The etiology of hypospadias in the majority of patients remains undefined, but is thought to involve both genetic susceptibility and environmental exposure to endocrine disruptors (West and Brenner, 1985; Baskin and Ebbers, 2006; Willingham and Baskin, 2007; Wang and Baskin, 2008; Kalfa et al., 2011). Treatment of hypospadias remains surgical, and multiple surgeries are often required for a functional and a cosmetically acceptable reconstruction (Lee et al., 2013). Patients with severe hypospadias are at risk for surgical complications that can lead to life long difficulties with urination, sexual function and psychological problems. Thus, hypospadias is a significant medical condition that consumes substantial health care resources.

An alternative approach to ameliorating hypospadias is prevention. If a genetically at-risk cohort could be identified and potentially causative environmental agents (endocrine disruptors) avoided, then the incidence of hypospadias could be reduced (Baskin et al., 2001a; Willingham and Baskin, 2007). For example, the incidence of hypospadias has been shown to be increased in families undergoing in vitro fertilization (Nordenvall et al., 2013), perhaps because progesterone is administered to maintain receptivity of the uterus to the embryo. Progestins have been implicated as a potential cause of hypospadias in both animal and human studies (Carmichael et al., 2005; Willingham et al., 2006a; Agras et al., 2007). Animal models of hypospadias have demonstrated a causal relationship between hypospadias and prenatal exposure to a variety of agents: estrogens, progesterone, Loratidine, “androgen blockers” (flutamide, finasteride, anti-androgenic fungicides [vinclozolin and procymidone], and phthalates) (Clark et al., 1993; Ostby et al., 1999; Kojima et al., 2002; Kim et al., 2004; Carmichael et al., 2005; Foster and Harris, 2005; Buckley et al., 2006; Willingham et al., 2006b; Ormond et al., 2009; Rider et al., 2009). The persistent question concerns the relevance of animal models to human hypospadias (Cunha et al., 2015b).

Estrogens are known to induce hypospadias in mice, and many studies use diethylstilbestrol (DES) as the teratogenic agent. The types of penile malformations seen in mice differ depending on whether DES treatment is prenatal or neonatal (Kim et al., 2004; Mahawong et al., 2014b, 2014a). It is likely, however, that a wider age range of DES exposure would reveal the “window of susceptibility” to adverse effects of DES, before and after which DES treatment may be without long-term teratogenic effects on individual elements within the developing external genitalia. Moreover, a thorough investigation of the effects of DES over a wide age range of treatment may (a) elucidate the morphogenetic mechanisms involved in generating abnormal penile morphology and hypospadias and (b) reveal those penile elements more (or less) sensitive on a temporal basis to developmental exposure to DES. Such an approach may also explain why certain effects of DES elicited and expressed during development resolve to normality in adulthood (Cunha et al., 2015b).

Hypospadias results from perturbation of normal penile development (Baskin et al., 1998), and thus can only be understood in the context of normal development of the penis, a complex organ with a precise anatomical patterning of its individual internal components. In humans, hypospadias refers to three related anomalies: (a) a urethral defect, (b) a preputial defect and (c) chordee (abnormal curvature of the penis). The abnormal urethral orifice may be situated distally in the glans, at mid-shaft, or in the perineum, indicative of mild, moderate or severe hypospadias (Cunha et al., 2015b). Associated with the defect in the urethral meatus is absence or hypoplasia of the corpus spongiosum as well as absence of the ventral aspect of the prepuce (Baskin et al., 1998).

Substantial differences in anatomy and development of the human versus the mouse penis necessarily translate to profound differences in the nature of hypospadias in these two species (Cunha et al., 2015b). In a general sense, hypospadias represents a perturbation of patterning of the elements constituting the penis, especially the positioning of the urethral meatus. While the various forms of human hypospadias are obvious on physical examination, hypospadias in the mouse is more subtle. First of all estrogen-induced mouse hypospadias does not involve “mid-shaft” malformations similar to that in humans. Indeed, prenatally estrogen-induced mouse hypospadias is characterized by subtle alteration in the (a) patterning of elements constituting the urethral meatus, namely the male urogenital mating protuberance (MUMP) and MUMP ridge, (b) an altered positioning of internal penile elements such as the os penis and urethral flaps relative to the urethral meatus and (c) malformation of the corpus cavernosum urethrae, the homolog of the human corpus spongiosum (Cunha et al., 2015b) (urethral flaps are projections of the corpora cavernosa urethrae into the urethral lumen) (Rodriguez et al., 2011). Even though mouse and human hypospadias are distinctly different, estrogen-induced mouse hypospadias exhibits certain morphogenetic homologies to human hypospadias based upon developmental processes common to both species (Mahawong et al., 2014b, 2014a; Cunha et al., 2015b). Whether penile defects elicited in mice by other agents (“androgen blockers” such as anti-androgen or 5a-reductase inhibitors, progesterone, phthalates, etc.) generate mid-shaft hypospadias remains to be seen.

Initial development of external genitalia in human and mouse embryos occurs identically in males and females and results in formation of the midline ambisexual genital tubercle, which is the primordium of the penis in males and the clitoris in females. In males, fetal testicular androgens elicit elongation of the genital tubercle. In both humans and mice a solid epithelial urethral plate forms (Fig. 1) that extends distally towards the tip of the genital tubercle. However, subsequent development of the urethral plate is radically different in humans versus mice. In humans, the urethral plate canalizes to form a wide urethral groove bounded laterally by urethral folds (Figs. 2, 3A, 5A and B) (Li et al., 2014). The human penile urethra forms as a result of midline fusion of the urethral folds, a process that begins proximally in the perineum and extends distally towards the glans penis (Figs. 2, 3, 4, 5A–C) (Li et al., 2014). Canalization of the urethral plate to form the urethral groove and subsequent fusion of the urethral folds to form the tubular urethral is particularly well illustrated in scanning electron micrographs (Fig. 3).

In mice the embryonic urethral plate extends to near the distal aspect of the genital tubercle, and canalizes to directly form most of the penile urethra (Fig. 5 D–F) (Hynes and Fraher, 2004a; Seifert et al., 2008). However, by birth the murine urethral plate is no longer observed within the distal aspect of the genital tubercle (Fig. 6A). Instead a ventral groove forms whose edges