Available online at www.sciencedirect.com
ScienceDirect
Reproductive Toxicology 23 (2007) 326–336
Reproductive Toxicology
www.elsevier.com/locate/reprotox

Developmental exposure of mice to TCDD elicits a similar uterine phenotype in adult animals as observed in women with endometriosis

Tultul Nayyar [1], Kaylon L. Bruner-Tran [1], Dagmara Piestrzeniewicz-Ulanska, Kevin G. Osteen *

Women's Reproductive Health Research Center, Department of Obstetrics & Gynecology, Vanderbilt University School of Medicine, 1161 21st Avenue S, MCN B-1100, Nashville, TN 37232, USA

Received 30 June 2006; received in revised form 28 August 2006; accepted 18 September 2006
Available online 30 September 2006

Abstract

Whether environmental toxicants impact an individual woman's risk for developing endometriosis remains uncertain. Although the growth of endometrial glands and stroma at extra-uterine sites is associated with retrograde menstruation, our studies suggest that reduced responsiveness to progesterone may increase the invasive capacity of endometrial tissue in women with endometriosis. Interestingly, our recent studies using isolated human endometrial cells in short-term culture suggest that experimental exposure to the environmental contaminant 2,3,7,8-tetracholorodibenzo-*p*-dioxin (TCDD) can alter the expression of progesterone receptor isotypes. Compared to adult exposure, toxicant exposure during development can exert a significantly greater biological impact, potentially affecting the incidence of endometriosis in adults. To address this possibility, we exposed mice to TCDD at critical developmental time points and subsequently examined uterine progesterone receptor expression and steroid responsive transforming growth factor-β2 expression in adult animals. We find that the uterine phenotype of toxicant-exposed mice is markedly similarly to the endometrial phenotype of women with endometriosis.

Keywords: Dioxin; TCDD; Progesterone; Progesterone receptor; TGF-β2; Endometrium; Endometriosis; Development; Fetal origin

1. Introduction

Polychlorinated dibenzo-*p*-dioxins, generally called dioxins, are a family of chlorinated aromatic hydrocarbons that accumulate as ubiquitous contaminants in our environment. In human populations, ingestion of contaminated food is the primary source of dioxin exposure [1–3]. These chemicals are resistant to degradation and, due to their lipophilic nature, bioaccumulate and biomagnify at higher levels within the food chain [4]. Among the numerous dioxin-like environmental contaminants, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is often considered to be the prototypical disruptor of steroid action within endocrine sensitive tissues, affecting steroid receptor levels as well as steroid metabolism and serum transport [5–7]. In addition to dramatically affecting the endocrine system, TCDD exposure also impacts the expression of multiple cellular signaling systems that regulate key elements of the immune system. For example, TCDD exposure can activate local inflammation through increased expression of tumor necrosis factor-α, interleukin-1β (IL-1β) and IL-6 [8,9]. Within either the endocrine or immune system, exposure to TCDD affects individual cell behavior by initially binding to the aryl hydrocarbon receptor (AhR; [10]) which rapidly forms a heterodimeric complex with ARNT (AhR nuclear translocator). The TCDD/AhR/ARNT complex can associate with dioxin response elements within the promoter of certain genes, subsequently exerting a wide variety of effects. The biological activity of various families of chemical toxicants on human health is diverse; however, toxicant exposure is generally classified into two models. The organizational model refers to chemical exposure of the embryo/fetus at very early stages of development, leading to both structural and functional changes in tissue development, whereas the activational model denotes activation of specific genes in adult tissue following acute or chronic exposure to environmental toxicants (reviewed by [11]). Although environmental toxicants have traditionally been classified separately as either endocrine-disruptors or immune-disruptors, a significant degree

* Corresponding author: Tel.: +1 615 322 4196; fax: +1 615 343 7913.
E-mail address: Kevin.Osteen@vanderbilt.edu (K.G. Osteen).
[1] These authors contributed equally and both should be considered as first authors.

0890-6238/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.reprotox.2006.09.007

of cross-talk occurs between these two systems and chronic inflammation has recently been associated with the development of numerous human diseases affecting multiple organ systems [12–14].

Not surprisingly, animal and human research has suggested that exposure to environmental agents may adversely affect the development and function of the reproductive system (reviewed by [15]). Notably, human exposure to TCDD has been suggested to negatively impact endometrial function during pregnancy, increasing the risk of spontaneous abortion [16]. More recently, evidence has begun to accumulate which suggests that TCDD exposure promotes the establishment of endometriosis [17,18], a disease resulting from the ectopic invasion of endometrial tissue, usually within the peritoneal cavity. Endometriosis is a disease of menstruating species; only humans and other primates exhibit naturally occurring endometriosis. Establishment of ectopic sites of endometrial growth can develop following retrograde menstruation due to the capacity of endometrial fragments to undergo a cancer-like invasive process. Although well-vascularized sites of ectopic endometrial growth can be extremely difficult to eliminate therapeutically using either surgical or medical approaches, endometriosis is estrogen-dependent and rarely persists post-menopausally in the absence of ovarian steroid production.

In opposition to the role of estrogen as a risk factor for endometriosis, progesterone may play a critical role in protecting a woman from the development and progression of this disease. For example, progesterone exposure during pregnancy, or exogenous therapy with various progestins, has been associated with disease regression in some women [19]. Nevertheless, reduced progesterone responsiveness has been noted in the eutopic endometrium of women with endometriosis, correlating with an altered expression of progesterone receptor (PR) isotypes [20]. In the endometrium of endometriosis patients, reduced levels of PR-B expression relative to PR-A likely explains the altered expression of progesterone-responsive genes that we and others have recently described during endometrial differentiation [20,21]. Additionally, diminished endometrial PR-B expression may be related to an increased sensitivity of stromal cells to locally produced proinflammatory cytokines [22,23] since this PR isoform has been shown to completely disappear within the inflammatory-like microenvironment that exists at ectopic sites of endometrial tissue growth [24]. As noted above, the eutopic endometrium of women with endometriosis exhibits alterations in the expression of a number of progesterone-regulated genes and proteins, including growth factors, cytokines and retinoid signaling proteins that are known to be critical for matrix metalloproteinase (MMP) regulation [25]. For example, the ability of progesterone or *all-trans* retinoic acid to down-regulate MMP expression in human endometrial tissue fragments, thereby reducing the invasive establishment of ectopic growth in nude mice, requires transforming growth factor-β (TGF-β) signaling [26–28]. Thus, due to reduced responsiveness to progesterone, blocking the establishment of experimental disease by endometrial tissues acquired from the endometrium of women with endometriosis requires TGF-β treatments in addition to progesterone and *all-trans* retinoic acid [27,29]. These results strongly suggest that reduced endometrial expression of progesterone-responsive genes and proteins, due to decreased expression of PR-B, may be an important contributing factor to an individual's overall risk for developing endometriosis. Although the cellular mechanism(s) associated with the development of reduced endometrial responsiveness to progesterone among endometriosis patients is not completely understood, our experimental data suggests that acute exposure of human tissue or cells to TCDD can dramatically reduce both PR-B expression and progesterone-mediated TGF-β2 expression, contributing to a more invasive endometrial phenotype in our experimental endometriosis model [20,27–29].

A possible role of environmental contaminants with dioxin-like activity in the development of endometriosis emerged with the demonstration that the incidence and severity of spontaneous endometriosis in rhesus monkeys was increased following dietary exposure to TCDD [30]. Although two recent epidemiologic studies demonstrated an increased level of dioxin-like compounds in the serum of women with endometriosis compared to disease-free women [31,32], other epidemiologic examinations have been less definitive [33,34]. Since human and animal exposures actually begin *in utero*, when toxicant sensitivity is greatest, developing a clearer understanding of the potential effects of TCDD and other environmental contaminants will likely require examining the impact of early, developmental exposure. In the current study, we provide further evidence, supporting our previously published work, of reduced endometrial responsiveness to progesterone in women with endometriosis. Additionally, we have examined the adult murine uterus following developmental exposure to TCDD and determined the impact of additional pre-pubertal and pubertal exposures to this toxicant, singly or in combination. We find that the altered expression of progesterone receptors and TGF-β2 that we observed in the endometrium of women with endometriosis occurs in the uterus of adult mice following TCDD exposure during critical periods of reproductive tract development and function.

2. Materials and methods

2.1. Acquisition of human tissues

Control endometrial tissues were acquired by Pipelle® (Unimar Inc., Wilton, CT) biopsy during the proliferative (days 9–12; $n = 8$) or secretory phase (days 13–17; $n = 7$) of the menstrual cycle from a donor population (age 18–45) exhibiting normal menstrual cycles and no history of endometriosis. Endometrial tissue from women with surgically confirmed endometriosis was also obtained by biopsy during the proliferative ($n = 4$) and secretory ($n = 4$) phases. Serum progesterone levels were assessed in order to confirm the cycle stage (proliferative ≤ 1.5 ng/mL; secretory > 1.6). Individuals with a recent (≤ 3 months) history of hormone therapy (i.e., oral contraceptives) were excluded. Biopsies were washed in prewarmed, phenol-red free Dulbecco's modified eagles medium/Ham's F-12 Medium (DME/F-12) (Sigma) to remove residual blood and mucous prior to culturing. Informed consent was obtained prior to biopsy and the use of human tissues was approved by Vanderbilt University's Institutional Review Board and Committee for the Protection of Human Subjects. Additional archived samples of formalin-fixed, paraffin-embedded endometrial tissues from women with surgically confirmed endometriosis were obtained from Vanderbilt University Medical Center's Histopathology Tissue Core.