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Dioxin and Endometrial Progesterone Resistance

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Abstract

Development of endometriosis likely requires multiple, interactive mechanisms involving both the endocrine and immune systems. Environmental toxicants, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), are of particular interest as potential contributory agents in the development of this disease because they can disrupt both systems. Nevertheless, defining the potential role that environmental exposure to TCDD plays in the development of endometriosis requires a better understanding of how this toxicant affects the biological processes that promote the disease. Although the disease mechanism(s) responsible for progesterone resistance in the endometrium of endometriosis patients remains speculative, our studies indicate that developmental exposure of mice to TCDD leads to a progesterone-resistant phenotype in adult animals that can persist for several generations. These studies and others underscore the importance of developing a greater understanding of the mechanisms of TCDD action that relate to reproductive disorders such as endometriosis.

Keywords

Endometriosis; TCDD; developmental exposure

In 1927, Dr. John Sampson theorized in a landmark publication that “endometrial tissue disseminated by menstruation is sometimes alive and will continue to grow if transferred to situations suited to its growth [such as] the peritoneum and surface of the ovary.”¹ Although the physiological purpose of menstruation remains uncertain, the mechanical process of endometrial tissue loss and bleeding places women and other primates at risk for the development of this disease. The true incidence of endometriosis is unknown but has been estimated to occur in ~5 to 10% of all reproductive age women in the United States.² However, because the diagnosis of endometriosis requires visualization by surgeons followed by histological confirmation, a significant number of patients likely remain undiagnosed. Although Sampson's retrograde menstruation theory remains the most widely accepted cause of endometriosis, retrograde menstruation is simply a *mechanical* risk factor, and thus the tissue-based etiology of this disease remains poorly understood. In general terms, endometriosis is a steroid-sensitive disease; however, defects of the immune system as well as genetic and epigenetic predisposition may play equally important roles in determining whether an individual will develop this condition.³ Over the past several decades, translational research groups have become increasingly concerned that environmental endocrine disruptors may play a role in the development of human diseases, including endometriosis.^{4–6} Endocrine-disrupting chemicals are of particular interest as potential contributory agents in the development of

endometriosis because they can alter steroid synthesis or action,⁷ lead to immune impairments,⁸ and can interrupt elements of reproductive function via epigenetic modification.⁹

The National Institutes of Health National Toxicology Program estimates that >80,000 chemicals have been released into our environment over the past few decades,¹⁰ and little data exist that truly assesses the potential risk of these toxicants to human populations. Among these toxicants, the endocrine disruptor 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and other polychlorinated dibenzo-p-dioxins, generally called dioxins, are members of a family of chlorinated aromatic hydrocarbons that accumulate as ubiquitous contaminants in our environment. These chemicals are resistant to degradation, and, due to their lipophilic nature, they bio-accumulate and biomagnify at higher levels in our food chain.¹¹ Thus, in human populations, ingestion of contaminated food is the primary source of TCDD exposure.^{12–14} TCDD accumulates within the human body, especially in areas of fat storage;¹⁵ it has an estimated half-life in humans of 11.5 years. For these reasons, TCDD is a significant tissue contaminant in the human breast, and breast milk samples have been found to contain very high levels of this compound, even in populations from poorly developed regions, reflecting the widespread transfer of dioxins and other toxicants from their point of origin.¹⁶ Very high levels of TCDD have been reported in breast milk in Belgium women,¹⁷ a country noted to have one of the highest incidences of endometriosis.^{18,19} Nevertheless, assessing the health risk of exposure to any single contaminant is difficult, and therefore the potential link between body burden of TCDD and the pathophysiology of endometriosis remains uncertain.¹⁹

To address this uncertainty, our research group has sought to define critical elements of steroid-mediated endometrial cell and tissue function that may be susceptible to disruption by TCDD and other environmental toxicants with similar activity. For example, in the absence of known toxicant exposure, we and others have noted reduced progesterone-sensitive gene and protein expression in endometrial tissue obtained from endometriosis patients compared with disease-free controls.^{20,21} Reduced progesterone sensitivity among endometriosis patients was particularly intriguing to us because we had previously linked in vitro TCDD exposure of human endometrium to disruption of progesterone action in an experimental endometriosis model.²² In this review, we describe recent experimental findings that further suggest a link between TCDD exposure and development of the progesterone-resistant endometrial phenotype.

PROGESTERONE-MEDIATED ENDOMETRIAL DIFFERENTIATION

In contrast to the influence of estrogen on the development of endometriosis, a woman's exposure to progesterone during pregnancy is a well-recognized negative risk factor for the development of endometriosis. Therefore, understanding the various ways an environmental toxicant that disrupts progesterone action might influence the development of endometriosis requires that we first consider critical elements of endometrial biology related to progesterone action. Although the earliest signs of menstruation are noted quite rapidly as a consequence of progesterone withdrawal, the stages of progesterone-mediated endometrial maturation occur relatively slowly over a period of nearly a week as the endometrium prepares for the invasive process of implantation and placentation.^{23–25} Establishing pregnancy is a biologically complex process, and progesterone must orchestrate the cooperative behavior of the multiple cell types that normally populate the maternal-fetal interface.^{26–29} Importantly, after successful establishment of the hemochorial placenta, progesterone continues to exert a critical influence on multiple endometrial cell types, and the stability of the maternal-fetal interface requires the action of this steroid throughout pregnancy. Thus it is the specific lack of continued progesterone support to the endometrium that triggers the initiation of the inflammatory-like process leading to tissue breakdown and menstruation.³⁰ Significantly, in the absence of pregnancy or medical intervention, this unique pattern of endometrial growth, differentiation,

and tissue breakdown can repeat itself >400 times over a woman's reproductive life. Assuming that Sampson's retrograde menstruation theory is correct, it seems remarkable that the development of endometriosis is not even more common. The simple fact that not all menstruating women have symptomatic endometriosis likely indicates that numerous factors, in addition to retrograde menstruation, are required for the development of clinically significant disease. Although retrograde menstruation allows for the mechanical transfer of endometrial tissue to the peritoneal cavity, it is the invasive behavior of the endometrial fragments within the peritoneal microenvironment that ultimately determines whether or not tissue fragments survive and grow at an ectopic site. Using an experimental model of endometriosis, our studies have shown that the progesterone-resistant endometrial phenotype observed in endometriosis patients is associated with an increased capacity for invading the peritoneal wall, obtaining a vasculature, and growing in the peritoneum of immunocompromised nude mice.⁴

Approximately 3 weeks of ovarian steroid priming is required to achieve optimal endometrial maturation and the opening of the "window of receptivity" requisite for successful nidation; therefore, menstruation marks a failed attempt to establish pregnancy. Following menstruation, estradiol production by ovarian follicles is responsible for a variable period of endometrial regrowth; however, the timing of endometrial maturation is more predictable in response to the increased production of progesterone by the post-ovulatory corpus luteum. Within the fundus region of the endometrium, researchers have shown that estrogen and progesterone receptors exhibit a distinctive, cell-specific distribution pattern that largely reflects the changing levels of ovarian steroid production.^{31,32} Within the functionalis region of the endometrium, ligand-bound steroid receptors mediate the transcriptional activation of specific genes coding critical proteins that subsequently promote changes in endometrial proliferation, morphology, and biochemistry across each phase of the menstrual cycle. Estradiol receptor levels peak during the late proliferative stage of the cycle, whereas progesterone receptor levels are highest somewhat later during the early secretory phase.³¹ Although in vivo and in vitro studies have shown that ovarian steroids can directly impact specific steroid-responsive genes in both endometrial stromal and epithelial cells,³³ the expression of progesterone receptors in epithelial cells have been shown to be significantly reduced as the endometrium matures near the time of implantation.³⁴

Loss of progesterone receptors in differentiating epithelial cells suggests that the specialized fibroblasts within the stroma of the endometrium are likely the primary cells remaining fully responsive to ovarian steroids during the midsecretory phase of the menstrual cycle.³⁵ Therefore, appropriate stromal-epithelial communication via secondary paracrine signals becomes increasingly critical for progesterone-mediated endometrial preparation for pregnancy.^{36,37} As relatively undifferentiated endometrial fibroblasts mature, they exhibit a remarkable transformation into decidual cells, producing a variety of progesterone-sensitive paracrine factors necessary for the appropriate differentiation of adjacent epithelial cells as well as other cells within the rich endometrial stroma.^{26,38,39} The complete cellular makeup of uterine decidual tissue is complex, and careful analysis has revealed interactive specialized fibroblast cells, an abundant vascular supply and numerous invasive cells of hemopoietic origin. Cells of hemopoietic origin residing within the decidua include leukocytes and macrophages that, in humans, can ultimately make up 40% of the decidual cell population.⁴⁰ The origins and specialized functions of various cells migrating into the human decidua remain to be determined due to the involvement of many different cell types as well as the ethical considerations that restrict the study of early human pregnancy. However, toxicant-mediated disruption of the differentiated function of the specialized endometrial fibroblasts as we have shown in vitro⁴¹ is likely a central component of the recognized ability of accidental TCDD exposure to disrupt human pregnancy.⁴²

TCDD AS A DISRUPTOR OF PROGESTERONE ACTION IN AN ENDOMETRIOSIS MODEL

Although human exposure to background levels of TCDD and other dioxin-like compounds cannot easily be avoided, the reproductive health consequence of chronic low-level exposure to these toxicants remains poorly understood. Among the numerous environmental toxicants in the dioxin family, TCDD (Fig. 1) is considered the most toxic and has been shown to be a prototypical disruptor of steroid receptor levels as well as steroid metabolism and serum transport.^{43–45} The biological effects of TCDD in responsive cells are mediated through high affinity binding to the arylhydrocarbon receptor, which subsequently forms an activated heterodimer complex with a structurally related nuclear transport protein upon ligand binding.⁴⁶ This activated complex can bind to specific DNA enhancer sequences known as dioxin response elements to affect the expression of specific genes.^{47,48}

Although the ability of experimental and accidental TCDD exposure to disrupt uterine function related to pregnancy in animals and humans is well known.⁴⁹ However, the primate study of Rier et al⁵⁰ was the first report suggesting a possible association between past exposure to TCDD and the subsequent development of endometriosis. In this study, Rier and colleagues reported the development of severe endometriosis in macaque monkeys previously exposed to dietary TCDD in a study originally designed to examine pregnancy outcomes. Several years after the completion of the original study, one of the animals was found to have died due to severe infiltrating endometriosis. Although the number of animals available for study was small, surgical assessment of the remaining monkeys suggested an association between severity of endometriosis and level of experimental TCDD exposure.⁵⁰ Although it is likely that the animals were also exposed to other toxicants in addition to TCDD,⁵¹ the initial study clearly raised the awareness of all endometriosis researchers to the potential contribution of environmental toxicants to this disease. Following the suggested role of TCDD in the development of endometriosis, a series of follow-up studies in cynomolgus monkeys^{52,53} and rodents^{22,54,55} continued to suggest an association between body burden of TCDD and the ectopic growth of endometrial tissue. However, no clear relationship between levels of TCDD exposure and endometrial growth was observed; at least one study reported that TCDD exposure may act to reduce the size of endometrial implants growing at ectopic sites.⁵² While toxicant-mediated ectopic endometrial growth observations are important, these animal studies did not specifically address the basic biological mechanism(s) that may link the action of TCDD to the establishment of ectopic disease. In particular, although TCDD is known to have both estrogenic and antiestrogen activity,^{56,57} our research suggested that disruption of progesterone action may be a more critical factor in determining the early establishment of peritoneal sites of endometrial growth.²²

As opposed to environmental toxicant exposure models conducted in animals, direct study of TCDD action in human tissues requires the development and application of various laboratory-based approaches. To study the influence of environmental toxicants on steroid-dependent development of endometriosis, our laboratory initially established an organ culture model in which small fragments of endometrial tissue are maintained in vitro for 2 to 3 days.³⁷ Organ culture models allow for the direct study of the multiple cell types and cell–cell relationships observed in vivo, and for many studies, they are superior to monolayer culture models of isolated cells for the study of basic tissue pathophysiology. In a collaborative study using our established organ culture system, Bofinger et al examined the impact of TCDD on human endometrial tissue expression of two cytochrome P450 enzymes, CYP 1A1 and CYP 1B1.⁵⁸ Both of these enzymes are expressed in extrahepatic tissues and are sensitive markers of TCDD exposure. Importantly, these enzymes are also involved in the metabolism of estradiol^{59,60} and potentially play an important role in both estrogen and progesterone action relative to the pathophysiology of endometriosis. In this study, we found that short-term exposure of human

endometrium to 10 nM TCDD increased the expression of both CYP 1A1 and CYP 1B1 mRNA and CYP1B1 protein over 72 hours of organ culture. Additionally, this study revealed another interesting component of endometrial physiology related to wound healing that may have relevance to peritoneal invasion by fragments of endometrium. We observed that CYP 1B1 mRNA expression increased at areas of re-epithelialization around the periphery of the organ cultures in the absence of TCDD exposure. These data, together with observations related to matrix metalloproteinase (MMP) expression, suggested to us that normal wound repair mechanisms and TCDD responses may involve similar mechanisms. For example, in newly established organ cultures undergoing active re-epithelialization, we have observed that the endometrial cells at the periphery of tissue fragments exhibit expression of MMP-7 in epithelial cells and MMP-3 in adjacent stromal cells, regardless of steroid exposure.⁶¹ Certainly, the wound-like behavior of endometrial fragments entering the peritoneal cavity may play a key biological role in the capacity of endometrial tissue to associate rapidly with the mesothelial cells lining peritoneal surfaces and subsequently break down and invade the extracellular matrix (ECM).

As opposed to most adult tissues, periods of tissue breakdown and ECM degradation and repair processes are normal components of the natural physiology of the human menstrual cycle. Using in situ hybridization localization, early studies by our research group demonstrated a cell-specific and menstrual cycle-dependent pattern of expression for several members of the MMP family in the cycling human endometrium.^{62,63} Looking at in vivo patterns of MMP-3 mRNA in stromal fibroblasts and MMP-7 mRNA expression in epithelial cells, we noted (1) cell-specific expression of mRNA for these MMPs during periods of estrogen-mediated cellular growth; (2) loss of MMP expression during endometrial maturation under the influence of progesterone; and, (3) re-expression of MMPs during ECM breakdown at menstruation. Additional in vitro studies by our laboratory and other groups have confirmed that progesterone is a potent inhibitor of MMP-3 and MMP-7 mRNA and protein expression in human endometrial stromal and epithelial cells, respectively.⁶⁴ However, following a brief in vitro exposure to TCDD, organ cultures of human endometrium collected from a control tissue donor population exhibit a loss of progesterone-responsive downregulation of MMP-3 and MMP-7.²² To determine experimentally whether the loss of progesterone-mediated down regulation of MMPs in our TCDD-treated organ culture model would affect the establishment of ectopic sites of endometriosis, we used our established mouse model.⁶⁵ In our experimental endometriosis model, fragments of human endometrium are collected during the growth phase of the menstrual cycle, treated with steroids in the presence and absence of toxicant and subsequently injected into the peritoneal space of nude mice. Using this model, we demonstrated that short-term exposures of human endometrial fragments to TCDD led to an increase in the secretion of MMP-3 and MMP-7 protein in vitro regardless of steroid cotreatment.²² These results in our model provided the first evidence that endometrial TCDD exposure can promote the establishment of experimental endometriosis by blocking the normally inhibitory effect of progesterone on MMP-mediated peritoneal invasion.⁶⁵ Additionally, examination of human endometrial organ cultures and isolated stromal and epithelial cells following exposure to TCDD further revealed that this toxicant likely disrupts the ability of progesterone to downregulate MMP expression by blocking both the expression of progesterone receptors⁴¹ and progesterone-dependent stromal-epithelial communication via transforming growth factor- β .²² We had previously shown that blocking the action of this growth factor significantly increases the secretion of MMP-3 and MMP-7 proteins in cocultures of endometrial stromal and epithelial cells.⁶⁶ Taken together, data obtained from our organ culture and coculture models as well as our experimental endometriosis model suggest that exposure of human endometrial cells and tissue to TCDD triggers a wound-like pattern of stromal-epithelial communication that effectively prevents normal progesterone-mediated regulation of the endometrial MMP system, and thus promotes the development of experimental endometriosis.

TCDD AND THE GENERATION OF ENDOMETRIAL PROGESTERONE RESISTANCE

As noted earlier, experimental primate studies as well as human observations have revealed that exposure to TCDD can disrupt pregnancy and lead to spontaneous abortion.⁶⁷ Pregnancy loss is not an unexpected reproductive consequence given that this toxicant is known to disrupt ovarian progesterone production⁶⁸ as well other important elements of endocrine and immune responses.⁶⁹ Whereas in vitro studies and some animal models continue to provide mechanistic clues suggesting that TCDD and similarly acting environmental toxicants may promote the development of endometriosis,^{4,70} epidemiological studies of human populations have not consistently confirmed such a link.⁷¹ Given that the development of endometriosis involves altered function of both the immune and endocrine systems, exposure to environmental toxicants is likely to be only one of several risk factors for this disease. A critical factor often overlooked in the study of environmental toxicants is the relative risk that exposures may play at different stages of life. Several recent studies in the field of environmental toxicology suggests that early life exposures to toxicants may epigenetically modify critical genes that can play a significant role in disease processes that only become evident later in adult life.^{9,72,73} In regard to progesterone action, using a murine model, we have shown that in utero exposure to TCDD impacts the sensitivity of the reproductive tract to future toxicant exposures as well as creating a uterine phenotype that exhibits insensitivity to progesterone.⁷⁴ Interestingly, following a combination of in utero and prepubertal TCDD exposure, adult mice can exhibit either infertility or a failure to sustain pregnancy to term.⁴ Even the surviving F2 and F3 offspring of TCDD-exposed mice (F1) are frequently found to exhibit reduced fertility, suggesting that toxicant-induced epigenetic changes may affect several generations. As shown in Fig. 2, the F1 generation of mice exposed in utero to TCDD exhibit changes in global methylation compared with unexposed animals; in addition to these global epigenetic changes, a specific loss of progesterone receptor (PR) protein expression was observed. Significantly, although 80% of F1 mice exhibited these changes, 20% of mice at the F5 generation continued to exhibit altered global methylation and reduced PR protein expression. As shown in Fig. 3, F1 mice that are able to attain pregnancy following in utero TCDD exposure are frequently found to exhibit a marked reduction in decidualization as noted on gestation day 7 (GD7) compared with control pregnant animals. These studies suggest that early-life exposures to TCDD can dramatically affect the function of the adult uterus, and that these affects can be passed to future generations. Certainly, if endometriosis in women is found to be a disease with developmental origins, studies attempting to link a woman's adult body burden of toxicants with her disease status may not reveal the true relationship between toxicant exposure and disease risk. Because experimental models of development toxicant exposures cannot be conducted in humans, we must rely on prospective data collected from accidental population exposures (i.e., Seveso, Italy) as well as carefully controlled laboratory-based animal studies.

SUMMARY/PERSPECTIVE

Although endometriosis is defined rather simply as the presence of both glandular and stromal elements of endometrial tissue at an ectopic site, the biological triggers for the development of clinically significant disease in only certain women remain unclear. Most investigators involved in endometriosis research suspect that multiple interactive mechanisms involving both the endocrine and immune systems are likely involved in the pathogenesis of this disease. Therefore, defining the potential role that environmental exposure to TCDD plays in the development of endometriosis will require a better understanding of how this environmental toxicant affects the biological triggers that promote the disease process. To approach such an understanding, our research group has used human endometrial tissues and cells and various in vitro models as well as an experimental endometriosis model. In initial experiments, we found that even brief TCDD exposure can disrupt progesterone action related to regulation of

several members of the endometrial MMP system. As we continued to explore the action of TCDD exposure on otherwise normal endometrial tissue and cells, we found that exposure to this toxicant disrupts MMP regulation by inhibiting normal progesterone-mediated patterns of stromal-epithelial communication. Given the established role of MMPs in endometrial tissue breakdown and repair, it is not surprising that TCDD also acts to increase the invasive behavior of human endometrium in our experimental endometriosis model. In general, we find that exposure of human endometrium to TCDD acts to promote a similar progesterone-resistant endometrial phenotype that we and others have observed in tissues acquired from women with endometriosis. Although the disease mechanism(s) responsible for progesterone resistance in the endometrium of endometriosis patients remains speculative, we have also found that developmental exposure of mice to TCDD leads to a progesterone-resistant phenotype in adult animals that can persist for several generations. At this juncture, whether experimental TCDD exposure models reflect a potential relationship of environmental toxicants to the patho-genesis of endometriosis in humans remains unclear. Nevertheless, laboratory-based studies with TCDD and other environmental toxicants are beginning to reveal important information about the potential role of the environment on not only endometriosis but other reproductive disorders as well. In particular, the potential that early-life exposure to environmental toxicants may affect our reproductive health for generations deserves more attention.

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2,3,7,8-tetrachlorodibenzo-p-dioxin

Figure 1.

Chemical structure of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD consists of two aromatic rings joined through a pair of oxygen atoms with four chlorine atoms attached at positions 2,3,7, and 8. Four hydrogen atoms are shown. Substituting additional chlorines in place of the hydrogen atoms creates other members of the dioxin family; thus up to eight chlorine atoms can be attached to the dibenzodioxin skeleton resulting in 75 possible chlorine-substituted isomers.

Figure 2.

(A–C) Immunohistochemical analysis of total endometrial progesterone receptor (PR) protein expression or (D–F) global methylation status (as determined by immunoreactivity to 5-methylcytidine) of (A, D) control mice, (B, E) F1 generation mice that were exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) during development (in utero, prepubertal) and F5 generation mice that were descended from an F1 mouse exposed to TCDD in utero and prepubertally. Original magnification, 200×. Uterine tissues from control mice exhibited abundant expression of both PR and immunoreactivity to 5-methylcytidine, whereas 80% of F1 mice with multiple TCDD exposures during development demonstrated markedly reduced expression of PR and global methylation as measured by 5-methylcytidine immunoreactivity.

By the F5 generation, only 20% of animals continued to demonstrate reduced endometrial PR expression and global hypomethylation.

Figure 3.

Pregnant C57/bl6 mice were sacrificed on gestation day 7 (GD7) and examined (A) grossly and (B–E) microscopically for implantation sites. Control mice typically exhibited eight or more implant sites that were readily identifiable on gross examination, whereas F1 dams with a history of in utero 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure had fewer and smaller implant sites. Microscopic examination of (B, D) control mice revealed a viable embryo, marked vascularization, and robust decidual response. In contrast, the F1 uterus exhibits a dramatically impaired decidualization response, reduced vascularity, and an embryo of poor quality. Original magnification (B, C) 40× and (D, E) 100×. Scale bars measure 200 μm (40×) and 80 μm (100×).