Dioxin and Steroid Regulation in an Endometriosis Model

Project Scope

The environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)—an unintentional by-product of several industrial chemical processes and waste combustion, and one of the more potent and well studied of a family of compounds referred to as dioxins—has been shown by numerous laboratories to disrupt estrogen action in reproductive tissues. TCDD also has been linked to the development in primates of endometriosis, a persistent, steroid-mediated disease that develops in 10-15 percent of women in industrialized nations, most often from the ectopic implantation of endometrial fragments that enter the peritoneum by retrograde menstruation. This link between TCDD and endometriosis, however, appears to be in conflict with TCDD's known anti-estrogenic effects, and thus mechanistic basis for the association between TCDD exposures and endometriosis in humans has been uncertain. Research under this grant was aimed at supplementing the epidemiological information in exposed human populations by studying the action of TCDD on specific cellular characteristics linked to the pathophysiology of endometriosis.

Grant Title and Principal Investigator

Dioxin and Steroid Regulation in an Endometriosis Model

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Key Findings and Implications

- The expression of matrix metalloproteinases (MMPs) was increased in endometriosis tissues, and is similar to observations in normal endometria that have been treated *in vivo* with TCDD.
- TCDD was found to prevent progesterone's normal suppression of MMP activity by blocking its ability to up-regulate TGF-β2 expression.
- TCDD disrupts the expression of the progesterone receptor isoforms A and B (PR-A, PR-B), resulting in a decreased PR-B/PR-A ratio. This is significant because a decreased PR ratio (increased PR-A; decreased PR-B) has been associated with both endometriosis and *in vitro* TCDD exposure.
- This grant demonstrated the benefits of an endometriosis model involving the ectopic implantation of human endometrial tissue in nude mice.

Publications include seven peer reviewed articles and a book chapter.

Project Period: December 1997 to December 2000

Researchers hypothesized that, as a consequence of TCDD exposure, progesterone-mediated regulation of matrix metalloproteinases (MMPs)—a group of enzymes that help regulate the site and extent of connective tissue remodeling throughout the body—may be disrupted. These researchers have previously demonstrated, using a nude mouse model to examine ectopic growth of human endometrium, that estrogen-associated MMP expression supports development of experimental endometriosis, while progesterone-mediated suppression of these enzymes inhibits the development of ectopic lesions. Although TCDD clearly has an impact on endometrial tissue function via interference or modulation of estrogen action, the ability of this agent to affect progesterone action is less understood. Thus, this grant sought to determine if and how TCDD can interfere with progesterone-mediated MMP regulation via modulation of local endometrial factors. Additionally, it sought to determine if this interference plays a role in the establishment or progression of TCDD-associated endometriosis.

The main objectives of this research were to:

- Compare the expression patterns of several cell-specific MMPs in endometriotic lesions obtained from women with those seen in experimental endometriosis in the nude mouse model;
- Identify the mechanisms by which TCDD exposure can disrupt progesterone-mediated regulation of endometrial MMPs; and
- Investigate the molecular role of TCDD in altering cell-type specific response of endometrial MMP expression within the environment of the peritoneal cavity.

Project Results and Implications

- Analysis of *In Vivo* MMP Expression Patterns: This study identified by *in situ* hybridization a highly variable expression pattern of MMP-3, MMP-7, and MMP-11 mRNA in endometriotic lesions obtained from different ectopic sites of disease growth. Although progesterone is normally a potent suppressor of cell-specific mRNAs for MMP-3, MMP-7, or MMP-11, expression of each gene, as well as protein secretion *in vitro*, was significantly increased in endometriosis tissues compared to the normal donor population. This finding is similar to observations in normal endometria that have been treated *in vivo* with TCDD.
- Cellular Mechanisms of TCDD Action: This set of experiments first explored the impact of TCDD on key elements of endometrial maturation during the menstrual cycle. Preliminary observations indicated that exposing human endometrial cell cultures to TCDD can prevent progesterone suppression of MMP-3 or MMP-7, which occurs *in vivo* during the progesterone-dominated secretory phase of the menstrual cycle. Because progesterone suppression of MMPs may be critical to prevention of experimental endometriosis, this study attempted to ascertain the specific cellular mechanism by which TCDD prevents progesterone suppression of MMPs. Progesterone induction of transforming growth factor (TGF-β2) is known to be a critical step in the pathway by which progesterone mediates suppression of MMP-7 expression, and blocking of TGF-β2 activity affects patterns of MMP-3 expression. During the first year of the grant, researchers were able to demonstrate that antibody treatments that block TGF-β2 action greatly reduced the ability of progesterone treatments to prevent experimental endometriosis. Furthermore, they demonstrated that *in vitro* TCDD exposure prevented the increase in endometrial TGF-β2 protein expression that normally follows exposure to progesterone. In subsequent experiments, researchers demonstrated that TCDD inhibition of TGF-β2 occurs at the level of the gene expression because progesterone-associated increases in TGF-β2 mRNA are also blocked by TCDD. Additionally, researchers found that disrupting TGF-β2 expression dramatically increases endometrial MMP expression during the establishment of experimental endometriosis. These results indicate that the ability of TCDD to prevent progesterone induction of TGF-β2 may play a critical role in the toxin's ability to alter the normal pathway by which progesterone regulates MMP expression.
- Molecular Mechanisms of TCDD Action. The aryl hydrocarbon (AhR) and aryl hydrocarbon receptor nuclear translocator (Arnt) genes are known to modulate progesterone activity. To investigate the effects of TCDD on endometrial cells at the molecular level, experiments were conducted to test the effects of AhR and/or Arnt expression on the activity of a progesterone-responsive promoter. AhR was shown to be present in stromal cells maintained with progesterone, but it could not be determined if progesterone or decidualization plays a role in AhR regulation. In a second series of experiments, the effect of TCDD exposure on endometrial stromal cell expression of progesterone receptor isoforms A and B (PR-A, PR-B) was investigated. The experiments showed that TCDD disrupts the expression of the two isoforms, resulting in a decreased PR-B/PR-A ratio. The results are significant because an altered PR ratio (increased PR-A; decreased PR-B) would lead to diminished progesterone action, likely affecting the expression of additional TGF-β signaling molecules. For example, the researchers found decreased PR-B/PR-A ratios in the endometria of women with endometriosis. Additionally, immunohistochemical analysis of endometria from women with endometriosis revealed a near absence of differentiation-related expression of TGF-β2, again reflecting the finding following *in vitro* TCDD exposure.

In summary, this grant has contributed substantially to the understanding of whether and how TCDD affects progesterone-mediated MMP regulation via modulation of local endometrial factors, as well as clarifying the role of TCDD in the establishment and progression of endometriosis. This grant also demonstrated the benefits of an endometriosis model involving the ectopic implantation of human endometrial tissue in nude mice.

Relevance to ORD's Multi-Year Research Plan

This project contributes primarily to two of ORD's Multi-Year Plan long-term goals, in particular (1) providing a better understanding of the science underlying the effects and assessment of endocrine disruptors and (2) supporting EPA's screening and testing program. More specifically, the results help elucidate the mechanisms/modes of EDC (TCDD) action so the effects can be extrapolated across classes of vertebrates and to critical biological stages that result in reproductive effects. This grant also refined and demonstrated the benefits of a nude mouse endometriosis model.

Investigators

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For More Information

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NCER Project Abstract and Reports
http://cfpub2.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/171/report/0
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