

Endocrine Disrupters and Endometriosis:

Is there a link?

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1.0 ABSTRACT

Endometriosis is a common gynecologic disease of unknown cause affecting approximately 10-15% of women of reproductive age and 50% of infertile women. Estrogen dependence and immune modulation are established features of endometriosis but do not adequately explain the cause of this disease. In recent years, evidence that environmental contaminants possess estrogenic activity has led to the hypothesis that exposure to hormonally active environmental contaminants contributes to the pathobiology of endometriosis. However, hospital based case-control studies have failed to provide compelling evidence for or against an association between environmental contaminant exposure and endometriosis. Results of animal studies however, suggest that it is biologically plausible for environmental contaminants to affect the pathobiology of endometriosis. Specifically, animal experiments implicate dioxin and dioxin-like compounds in this disease. Herein the literature linking environmental contaminants with endometriosis is reviewed and the link with endocrine disruption discussed.

2.0 INTRODUCTION

Endometriosis is an estrogen-dependent disease defined as the growth of endometrial glands and stroma at extra-uterine sites and is present in approximately 10% of reproductive-aged women (Wheeler, 1992). Although the cause of endometriosis is unknown, several mechanisms have been proposed to account for the implantation of endometrial cells outside of the uterus including coelomic metaplasia, vascular transport and retrograde menstruation of endometrial cells. Back-flow of menstrual contents into the pelvic cavity is thought to afford endometrial cells the opportunity to implant outside

of the uterus and is the most widely accepted theory for the cause of endometriosis (Sampson, 1922). A number of lines of evidence lend support to this theory. First, endometriosis is found more often in women with outflow defects of the uterine cavity (Olive and Henderson, 1987). Secondly, partial obstruction of the cervical os in baboons resulted in endometriosis induction rates of 100 % within 3 months (D'Hooghe et al., 1994). Although retrograde menstruation or bleeding into the peritoneal cavity during menstruation is widely accepted as a major contributing factor in the pathogenesis of this disease, it is a common phenomenon occurring in approximately 70 to 90 % of women (Halme et al., 1984). Hence, factors other than access of endometrial contents to the pelvis via retrograde menstruation are thought to contribute to the pathogenesis of endometriosis.

Endometrial cells destined to become endometriotic implants are thought to be different from normal endometrial cells, a notion that is supported by a number of distinct observations. Endometrial cells from women with endometriosis survived transplantation in athymic nude mice longer than normal proliferative phase endometrium from women without endometriosis (Bruner et al., 1997) suggesting that these cells are functionally distinct. There are several important changes in endocrine regulation of the endometrium that are proposed to be important in the pathobiology of endometriosis. Aromatase gene expression levels have been demonstrated to be greater in endometriotic cells compared to eutopic endometrium from women with endometriosis, whereas expression was absent in the eutopic endometrium of disease-free women and non-endometriotic pelvic tissues from women with endometriosis (Noble et al., 1996). Aromatase is the rate-limiting

enzyme responsible for the conversion of androgens (e.g. testosterone and androstenedione) to estrogens. It has also been shown (Zamah et al., 1984) that the endometrium of women with endometriosis metabolize estrogen less readily due to lower levels of the enzyme 17β -HSD-II, the enzyme that converts estradiol to estrone, a less potent form of estrogen. Taken together, increased aromatase expression and lower levels of 17β -HSD-II are proposed to create a high local estrogen environment in the ectopic endometrium that favors the survival of endometrial stromal cells and potentially endometriosis. While the critical event(s) or biochemical change(s) that ultimately leads to establishment of endometriosis remains an enigma, dysregulation of estrogen metabolism or signaling in the endometrium is likely to play an important role.

3.0 EPIDEMIOLOGY OF ENDOMETRIOSIS: Although endometriosis is a common gynecologic problem, surprisingly little is known about the risk factors contributing to this disease. Associations between endometriosis and various factors including reproductive health, personal habits, body characteristics, immunological, and genetic factors have been described. Of the sociodemographic factors thought to be important in the development of endometriosis, age is the only characteristic for which a consistent positive relationship has been observed. This association peaks among women aged between 40-44 years (Vessey et al., 1993). There also appears to be an increased risk in individuals from higher socioeconomic stratas. This association can, however, be explained in part by selection bias since these individuals are more likely to seek medical attention and surgical management. Additionally, delayed child-bearing or nulliparity in this group of women may also account for the observed relationship between

endometriosis, age and socioeconomic status. Nevertheless, we propose that there are two key risk factors that are associated with endometriosis. Specifically, conditions that increase the opportunity for retrograde menstruation and increased circulating levels of estrogens will increase a woman's risk for endometriosis. For example, shorter cycle lengths, longer duration of flow, delayed childbearing, and reduced parity are all associated with an increased exposure to estrogen and therefore increased risk of endometriosis (Eskenazi and Warner, 1997). Furthermore, endometriosis is more likely to be found in women with increased peripheral body fat compared to women with central obesity. This is thought to occur because women with increased peripheral body fat have elevated estrogen levels due to peripheral conversion of androgens to estrogens (Eskenazi and Warner, 1997). In contrast, lifestyle habits that appear to decrease estrogen levels have been associated with a decreased risk of endometriosis. These include smoking and exercise, which are thought to increase estrogen turnover by the liver (Eskenazi and Warner, 1997).

3.1 Human Exposure to Environmental Contaminants and Endometriosis: A small number of hospital-based case-control studies have been designed to explore the association between endometriosis and exposure to environmental contaminants. Exposure to polychlorinated biphenyls (PCBs) and dioxins has been loosely linked with endometriosis (Gerhard and Runnebaum, 1992; Koninckx et al., 1994). In the former study (Gerhard and Runnebaum, 1992), serum levels of PCBs 138, 153 and 180 were elevated compared to women without endometriosis. A letter to the editor (Koninckx et al., 1994) noted that the levels of dioxins in Belgium are among the highest in the world

and the incidence of endometriosis is also higher in this country than elsewhere. A positive association between endometriosis and dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD) exposure was reported in a single case control study in which 44 women with endometriosis were compared with 35 age-matched women with tubal infertility (Mayani et al., 1997). In this study, one control subject had a detectable level of TCDD whereas 8 women of 44 had detectable levels. While an OR of 7.6 was obtained, the 95% CI included unity (0.87 - 169.7) and thus it cannot be concluded that dioxin exposure alone is associated with endometriosis. It is possible; however, other chemical contaminants that act through a common mechanistic pathway may be involved. A weakness of this study is the failure to control for obstetrical history. Since a full term pregnancy and breast-feeding are thought to decrease body burdens of lipophilic environmental contaminants, it is not possible to determine if prior pregnancies may have accounted for the low number of detections observed in this study. In another study (Eskenazi et al., 2000) the association between endometriosis and exposure to dioxin has been investigated in women living in Seveso, Italy. Results of the Seveso Women's Health Study (SWHS) reveal a non-significant doubling of risk.

In another study, no association between endometriosis and PCB specific congeners and organochlorine pesticide concentrations could be found in a case-control study of 86 women with endometriosis and 70 controls matched for the indication of laparoscopy (Lebel et al., 1998). In this study, congener specific PCB analysis was conducted as a surrogate for TCDD, which is thought to co-migrate with the PCBs. While the exposure assessment taken in this study permitted a broader assessment of the exposures that could influence the pathogenesis of endometriosis, the absence of a truly

unexposed control population and sample size limitations are both factors that may have prevented detection of an association between endometriosis and exposure. Similarly, another team of investigators was unable to find an association between endometriosis and exposure to dioxins and furans in a study involving 15 control subjects and 15 cases (Boyd et al., 1995).

In a recent study (Pauwels et al., 2001) the association between endometriosis and environmental contaminant exposure was evaluated using the CALUX assay to determine the level of dioxin-like activity of chemicals present in the serum. In this study serum samples were collected from 42 women compared to 29 women without endometriosis. However, as in the previous studies no relationship between endometriosis and dioxin-like activity was found. The authors however note that this was a pilot study and a minimum of 100 subjects in each group would be needed to detect a significant association. Hence, all of the case-control studies described in the literature to date are relatively small, and thus may not have the statistical power to detect differences if they were indeed present. Therefore, the human data at present neither confirm nor refute the hypothesis that environmental contaminants play a role in the pathobiology of endometriosis.

4.0 Biological plausibility:

4.1 Non-human primate studies – Endometriosis was accidentally discovered in a reproductive/developmental toxicology study, in which rhesus monkeys were treated with TCDD (Rier et al., 1993). The monkeys were treated with TCDD for four years and several years after the study was initiated several monkeys developed endometriosis.

Laparoscopies were performed approximately 6 years after the completion of TCDD treatment to investigate the potential relationship between TCDD treatment and endometriosis. A dose-dependent increase in the incidence and severity of endometriosis was found in this study. However, Rier and coworkers (Rier et al., 2001) have subsequently reported the results of residue analysis of the serum for TCDD and PCB specific congeners from the monkeys involved in the original endometriosis study (Rier et al., 1993). Residue analysis revealed that the incidence and severity of endometriosis was not correlated with serum TCDD levels. However, elevated serum levels of 3,3',4,4' tetrachlorobiphenyl (TCB), 3,3',4,4',5-pentachlorobiphenyl (PnCB) were associated with a higher prevalence of endometriosis whereas the severity of endometriosis was correlated with the serum concentration of 3,3',4,4'-TCB. Both of these coplanar PCBs are believed to act through the aryl hydrocarbon receptor (AhR). The majority of the toxic effects of TCDD are thought to be mediated through the AhR (Mimura and Fujii-Kuriyama, 2003). A recent study (Ohtake et al., 2003) has demonstrated that low concentrations of TCDD bound to the AhR can interact with estradiol and its receptor to induce an estrogen-mediated proliferative effect in the mouse uterus. Consequently, it is suggested that AhR ligands together with or in addition to TCDD can act via an endocrine pathway and are important in the pathophysiology of endometriosis.

In another reproductive/developmental toxicology study, monkeys were exposed to a commercial PCB mixture, Arochlor 1254, over a five-year period. As in the Rier et al., (1993) study several monkeys developed endometriosis raising concern that treatments were inducing this disease. Therefore, the monkeys were examined either by laparoscopy (control and high dose monkeys) or necropsy for evidence of endometriosis

and adenomyosis (growth of endometrial epithelial and stromal cells in the muscle wall of the uterus). Rhesus monkeys treated with the commercial PCB mixture, however failed to show any relationship between the incidence and/or severity of endometriosis and PCB dose (Arnold et al., 1996). It is proposed that the PCB mixture used in this study (Arnold et al., 1996) possesses low dioxin like activity and thus would not be expected to have similar effects to TCDD.

In another study (Yang et al., 2000) the effect of TCDD on surgically induced endometriosis was investigated. The doses of TCDD employed in this study were selected to be equivalent to those used by Rier and colleagues (Rier et al., 1993) and expanded to include one lower dose level. Interestingly, this study revealed a bimodal effect of TCDD on the maximum and minimum implant diameter. Maximal and minimum diameters of the lesions were significantly greater in the 25 ppt dose group compared to the controls and reduced in the 1 ppt group compared to controls. Over the course of the study implants were found to regress in all treatment groups but significant differences were not found until the completion of the study at one-year post implantation. Survival of ectopic endometrial implants was also affected by TCDD treatment with survival being significantly enhanced at the 5 and 25 ppt level compared to the controls. Consequently, this study supports and extends the findings of the earlier study in rhesus monkeys (Rier et al., 1993). Furthermore, these data suggest that duration of exposure to TCDD and by implication other AhR ligands must be lengthy before effects on endometrial implant survival and proliferation can be discerned.

4.1 Rodent studies: Surgical induction of endometriosis in rodents has been used to investigate the effect of environmental contaminants on the establishment of endometrial implants, survival of existing implants, and mechanism of contaminant action. In two separate studies (Cummings and Metcalf, 1995a,b) involving mice and rats autologous uterine implants were found to be estrogen dependent and responsive to an estrogenic contaminant methoxychlor. In another study ovariectomized mice with autotransplanted uterine strips were used to demonstrate that administration of 4-chlorodiphenyl ether, an estrogenic compound, could facilitate implant growth (Yang et al., 1997). The rodent studies therefore reveal that estrogenic contaminants affect the survival and proliferation of autologous uterine implants in rodent models making these suitable models for testing chemical contaminants for potential activity in this disease. Accordingly, these models have been used to explore the effect of TCDD on the growth of uterine implants which has been shown to enhance the growth of implants (Cummings et al., 1996). Administration of 0, 3 or 10 µg TCDD/Kg on gestational day 8 and then again three weeks before surgical induction of endometriosis in both the rat and the mouse resulted in an increase in implant size (Cummings et al., 1996). In another study (Johnson et al., 1997), endometriosis was surgically induced in mice and the animals were treated with 0, 1, 3 or 10 µg TCDD/Kg; 3 or 30 mg PCB 153; 100, 300 or 1000 µg PCB 126/Kg; 10, 30, 100 µg 4-PeCDF/Kg; or 2 or 20 mg 1,3,6,8-TCDD by gavage, five times 3 weeks apart. Following necropsy 3 weeks after the final dose, 2,3,7,8-TCDD and 4-PeCDF significantly enhanced the growth of endometrial lesions whereas PCB 126 and the highest dose of TCDD were without effect on growth of the endometrial lesions. Moreover, the non-dioxin-like compounds were without effect on lesion growth. These

data suggest therefore that it is the dioxin-like compounds that possess the ability to modulate the pathophysiology of endometriosis. In contrast to these findings ovariectomized and estrogen-replaced mice, treated with 10, 50 and 100 ng TCDD/Kg/day resulted in inhibited growth of previously established implants (Yang and Foster, 1997). Taken together these data suggest that the dose of TCDD and that the length of time that the implants are exposed to TCDD are also important.

Rodent models of endometriosis are complicated by a number of significant limitations. The estrous cycle in rodents is significantly shorter than in humans and is characterized by endocrinological differences such as an abbreviated luteal phase compared to humans. Moreover, circulating levels of estradiol in rodents are markedly lower than in humans. Therefore, extrapolation of rodent study results to humans is difficult and fraught with uncertainty concerning differences in the physiology of rodent uterine tissue vs. human endometrium.

5.0 SUMMARY AND CONCLUSIONS: The findings from epidemiological studies are inconsistent and thus these studies neither support nor disprove the hypothesis that exposure to environmental contaminants are linked with an increased risk for endometriosis. However, the biological plausibility of the association between exposure to environmental contaminants and endometriosis is provided by several non-human primate and rodent studies. Unfortunately, the mechanism of action underlying the observed effects remains to be determined and an endocrine mechanism has yet to be demonstrated. Therefore, while the evidence linking exposure to environmental contaminants and endometriosis is weak at present, evidence of biological plausibility for the hypothesis is strong and thus further study of the association between exposure to

environmental contaminants and endometriosis together with investigation of underlying mechanisms is needed.

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