ARE DIOXINS ASSOCIATED WITH SEVERE ENDOMETRIOSIS IN WOMEN?

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Introduction

Endometriosis is a gynecological disorder characterized by the presence of functional endometrial glands and stroma outside the uterus[1], which causes internal bleeding, inflammation and scarring, and often leads to pelvic pain and infertility[2]. Endometriosis has been estimated to affect as many as 10% of women of reproductive age[1].

It has been proposed that environmental compounds with endocrine-disrupting or estrogen-like activity, such as the highly persistent and toxic polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs), may be involved in the pathogenesis of endometriosis[3]. Dioxins and PCBs are lipophilic compounds which preferentially bio-accumulate in the food chain[2]. Over 90% of the total exposure to these compounds is due to food consumption, with animal products being the major contributors [4]. Exposure to these chemicals results in a variety of toxic effects including immunologic, neurochemical, and neurotoxic[5]. Seventeen dioxins and 12 dioxin-like (DL) PCBs bind to the aryl hydrocarbon receptor (AhR), which mediates most of their toxic effects and activates several genes, and therefore are classified relative to the most toxic dioxin, 2,3,7,8-TCDD, a known carcinogen, and given a toxic equivalency factor (TEF)[2].

The association of dioxins and PCBs to endometriosis has been supported by several experimental animal studies[4,6]. These studies prompted a series of hospital based endometriosis studies to investigate the association between dioxins and PCBs and endometriosis, which resulted in conflicting results[7]. Important differences in study design, patient selection, analytes assessed and assay methods affect the comparability of the results[8].

The aim of this study was to determine whether an association between dioxins, DL-PCBs and marker PCBs and deep-infiltrating endometriosis (DIE) exists by analyzing blood serum and adipose tissue samples from women (n=20) who have been surgically confirmed to have DIE and a group of control women (n=20) in which endometriosis has been surgery discarded as a diagnosis. This study is remarkable as both adipose tissue and serum samples have been analyzed, whereas most other human studies have analyzed only blood samples. A preliminary study was presented at Dioxin 2008.

Materials and Methods

Twenty patients with severe DIE and twenty control women, all receiving medical care from the Hospital Clinic in Barcelona, Spain, were recruited to be part of this study. All of the patients were surgically confirmed to have deep-infiltrating rectovaginal endometriosis by laparoscopy. The women from the control group were under the age of 40 years, healthy, had no pregnancies, demonstrated no malignancy and were surgically confirmed to not have endometriosis. Adipose and serum samples from each patient were obtained and kept frozen until sample analysis.

In this study, analytes were extracted from serum samples by solid phase extraction with C18 and Na_2SO_4 cartridges, while the adipose tissue samples were solid-liquid extracted by refluxing for 24 hours with toluene and cyclohexane. All samples were spiked with known amounts of mixtures of $^{13}C_{12}$-PCDD/Fs (EPA-1613LCS,

Wellington Laboratories Inc., Guelph, Canada), $^{13}C_{12}$-DL-PCBs (WP-LCS, Wellington Laboratories Inc., Guelph, Canada), and $^{13}C_{12}$-PCBs (MBP-MXE, Wellington Laboratories Inc., Guelph, Canada). Treatment with sulphuric acid was applied to remove organic components, fat and other interfering compounds, while purification was accomplished with the use of multilayer silica, basic alumina and carbon adsorbents. Fat determinations of the serum samples were performed by enzymatic methods.

Instrumental analyses were performed on a 6890N GC System gas chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) fitted with a 60m x 0.25 mm i.d. x 0.25 um film thickness DB-5ms fused silica column (J&W Scientific, CA, USA) coupled to a high resolution mass spectrometer controlled by a MassLynx data system. Positive electron ionization (EI+) operating in the SIM mode at 10000 resolving power was used. All samples were quantified based on the isotope-dilution method. The criteria for ensuring the quality of analysis include the application of quality control (QC) and quality assurance (QA) measures.

Results and Discussion

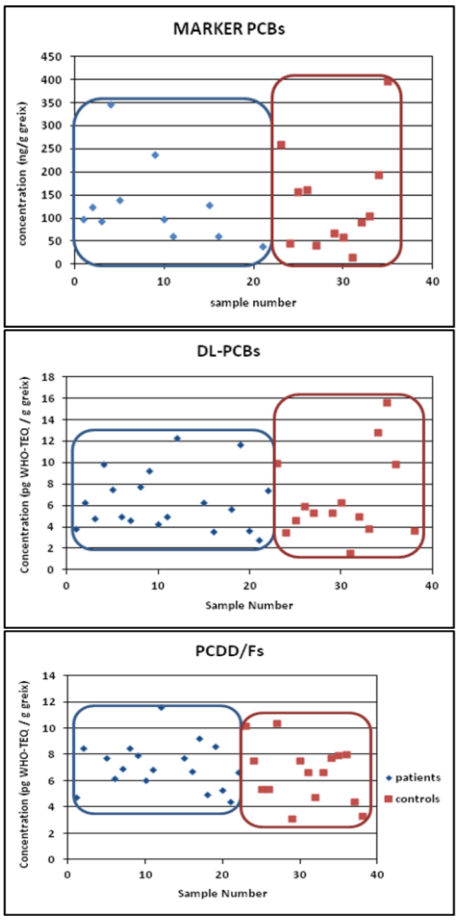

Figure 1. PCDD/Fs, DL-PCBs and marker PCB data for endometriosis patients and control women in adipose tissue.

Levels of PCDD/Fs, DL-PCBs and marker PCBs were determined in serum and adipose tissue of a group of 20 women with severe endometriosis and a control group of 20 healthy women. The level of marker PCBs in adipose tissue samples for patients ranged from 40-347 ng/g fat, with a median ± standard error (SE) of 124 ± 25 ng/g fat. This value was higher than for the control group that had a median ± SE of 92 ± 31 ng/g fat. Although the control women have a lower median level than the endometriosis patients, the concentration range is slightly higher with a minimum value of 16 ng/g fat and a maximum value of 397 ng/g fat. The same is true for DL-PCBs. The median concentration for patients was 5.6 ± 0.6 pg WHO-TEQ/g fat and 5.4 ± 1.1 pg WHO-TEQ/g fat for controls. The minimum and maximum values for patients and controls were 2.8-12.3 pg WHO-TEQ/g fat and 1.5-15.6 pg WHO-TEQ/g fat, respectively. For PCDD/Fs, the median values were very similar. The controls have a slightly higher value of 7.1 ± 0.6 pg WHO-TEQ/g fat compared to the patients who have a value of 7.0 ± 0.4 pg WHO-TEQ/g fat. Also, the concentrations range for patients (4.4-11.6 pg WHO-TEQ/g fat) was slightly lower than for controls (3.1-11.4 pg WHO-TEQ/g fat). The concentrations of PCDD/Fs, DL-PCBs and marker PCBs in adipose tissue for patients and controls are plotted in Figure 1. The median values show a trend of higher concentrations for endometriosis patients compared to controls, but if all the data is examined in detail, it is difficult to clearly distinguish the two groups.

For the serum samples, the median ± SE for PCDD/Fs and DL-PCBs in patients were 12.8 ± 2.7 pg WHO-TEQ/g fat and 8.7 ± 2.0 pg WHO-TEQ/g fat, respectively. These values were higher in serum than in adipose tissue samples, although for marker PCBs, the serum samples had a lower median concentration of 113 ± 16

ng/g fat. The serum samples from the control women have yet to be analyzed, which could provide further insight, especially for comparability, as most human studies on endometriosis have analyzed blood samples.

The PCDD/F, DL-PCB and marker PCB profiles in serum and adipose tissue from patients with endometriosis and control subjects were similar. PCDD/Fs profiles showed major contributions of 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD and 2,3,7,8-TCDD in pg WHO-TEQ/g fat. The DL-PCBs in abundance were PCB-126, PCB 156 and PCB 118 in pg WHO-TEQ/g fat, and the highest values for marker PCBs were PCB 180, PCB-153 and PCB 138.

In summary, the levels of DL-PCBs and marker PCBs in adipose tissue show higher median values in women with endometriosis compared to control women in this study, while PCDD/Fs have similar median values. Looking at all the adipose tissue data, it is difficult to distinguish the patients from controls. Serum samples have higher PCDD/F and DL-PCB concentrations for patients than in adipose tissue, although the control serum data is yet to be determined. As endometriosis is an understood disease, further studies are necessary to determine the factors that play a role in its etiology.

Acknowledgements

We would like to thank Jordi Sauló for his help with the instrumental analysis.

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