ANALYSIS OF PCDD/Fs AND DL-PCBs IN HUMAN SERUM AND ENDOMETRIOSIS : PRELIMINARY STUDY IN SPAIN

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Introduction

There is evidence that polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (DL-PCBs) may adversely affect the health of wildlife and humans[1]. Humans are daily exposed to complex mixtures of PCDD/Fs and DL-PCBs mainly via trace amounts present in food[2]. A variety of toxic effects in experimental animals exposed to these chemicals, including immunologic, neurochemical, neurotoxic, carcinogenic and endocrine changes have been reported[3].
Endometriosis is an estrogen-dependent gynecological disease characterized by the presence of ectopic endometrium in the peritoenal cavity which causes internal bleeding, inflammation, scarring and often leads to infertility[4,5]. Despite the widespread occurrence of this disorder little is known about its pathophysiology. Nevertheless, it has been suggested that endocrine disruptors, such as PCDD/Fs or DL-PCBs, could play a role in the onset of the growth of endometriosis[4]. For instance, some studies reported higher levels of these compounds in serum samples of women with endometriosis than in controls[6].
In the present study, a case-control design over women with endometriosis and controls has been undertaken. This work constitutes the first preliminary study in Spain in which the link between PCDD/F and DL-PCB exposition and endometriotic diseases is evaluated.

Materials and Methods

Five patients, aged 25-35 years, with severe deep endometriosis were selected for this preliminary study. In adition, volunteer women from between 22 and 70 years old were selected as a control subjects. They were divided in two control groups depending on the age; from 22 to 44 and 45 to 70 years old. Motherhood was not a decisive factor to select the volunteers for control subjects and endometriosis patients in this study. Blood samples were collected individually in a vaccum system tube and the serum was obtained within 24 hours after collection. Serum was kept frozen at -20ºC until PCDD/F and DL-PCB analysis.
Samples were spiked with known amounts of mixtures of $^{13}C_{12}$-PCDD/Fs (EPA-1613LCS), $^{13}C_{12}$-DL-PCBs (WP-LCS) and marker $^{13}C_{12}$- PCBs (MBP-MXE), all of them obtained from Wellington Laboratories Inc. (Guelph, Canada). After that, sample extraction was performed by liquid-liquid extraction. Next, a treatment with sulphuric acid was applied to remove organic components, fat and other interfering substances. Finally, the extracts were concentrated and transferred to n-hexane prior to the purification step. Details on the clean-up procedure based on the use of the Power PrepTM system (FMS Inc., MA, USA) is described elsewhere[7]. GC coupled to high resolution mass spectrometry (HRMS) was used for the final instrumental analysis. All 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 µm film thickness DB-5ms fused silica column (J&W Scientific, CA, USA) coupled to an Autospec Ultima NT HRMS (EBE geometry) (Micromass, Manchester, UK) controlled by a MassLynx data system. Positive electron ionization (EI+) operating in the SIM mode at 10000 resolving power was used. Quantification was carried out by isotopic dilution. Fat determination was performed by enzymatic methods[8,9]. The criteria for ensuring the quality of dioxin analysis include the application of quality control (QC) and quality assurance (QA) measures, such as a continuous monitoring of laboratory contamination based on the determination of a blank sample covering the whole analytical procedure, including extraction, clean-up and quantification[10].

Results and discussion

Levels of PCDD/Fs, DL-PCBs and marker PCB were determined in the two control groups and subjects with severe deep endometriosis. In Table 1 concentration of PCDD/Fs, DL-PCBs and marker PCBs in serum samples from women affected and non-affected (control) by endometriosis are summarized. Control samples revealed for PCDD/Fs an average concentration of 17.49 and 27.24 pg WHO-TEQ g^{-1} fat weight (f.w.) for control group 1 and 2, respectively. Similarly, levels of DL-PCBs were 11.76 and 19.32 pg WHO-TEQ g^{-1} f.w., for control groups 1 and 2, respectively. As a result, the total sum of marker PCBs ranged between 90.28 for women from 22 to 44 and 182.83 ng/g f.w., for women from 45 to 70 years old. As expected, an increase on the levels of the studied families of organochlorine pollutants could be correlated with the women's age.

On the other hand, samples collected from patients with endometriosis presented higher concentration of PCDD/Fs than control samples with similar age (22 to 44 years), with values varying between 32 and 88 pg WHO-TEQ g^{-1} f.w. These values are comparable to those found in control samples from older women (from 45 to 70 years old). Similary, comparable results were obtained for DL-PCBs for patients with endometriosis, between 25 and 34 pg WHO-TEQ g^{-1} f.w., and control groups of 45 to 70 years. Only samples M1 and M4 (9.29 and 5.97 pg WHO-TEQ g^{-1} f.w., respectively) showed similar values to control group of similar age (22 to 44 years). However, results obtained for PCBs markers were two to three times higher than both control groups, except for sample M1 that showed a similar value than the control group between 20 and 44 year. A comparison between PCDD/F profile of sample M3 and control group of 22-44 years is depicted in Figure 1. In general, congener distribution of PCDD/Fs in serum samples from subjects with severe deep endometriosis is similar to the profile observed in control subjects. It must be indicated that the percentage of the lowest chlorinated compounds, particularly tetra- to hexachlorinated congeners, was aproximately the 90% of the total WHO-TEQ, and in addition, PeCDD and 2,3,4,7-PeCDF contributed aproximately to the 52% of the total WHO-TEQ. For DL-PCBs and PCBs markers the profile became similar between subjects with endometriosis and control subjects. As expected for DL-PCBs, major contribution to the total concentration (pg g^{-1} f.w.) was due to PCB 118, which represented more than 50%, followed by PCB 15, with a relative percentage near to 15%. For PCBs markers the major contribution was that of congeners with higher chlorinated compounds, PCB 153, 158 and 180.

In summaryconclusion, the results obtained in this study show that PCDD/F, DL-PCB and PCB indicator levels in serum from severe deep endometriosis subjects have higher levels than the control subjects. This fact suggested a possible association of PCDD/Fs content with the occurrence of endometriosis. Since endometriosis is an understood disease, further studies are necessary to determine the factors that play a role in its etiology.

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Table 1. Concentrations of individual PCDD/F (pg g^{-1} f.w.), DL-PCB (pg g^{-1} f.w.) and marker PCB (ng g^{-1} f.w.) congeners and total levels, expressed in pg WHO-TEQ g^{-1} f.w., of these contaminants in serum samples.

conc. (pg g^{-1} f.w.)	M1	M2	M3	M4	M5	CONTROL 1 age 22-44	CONTROL 2 age 45-70
2,3,7,8-TCDD	<2.86	3.70	<6.54	<2.55	<3.80	<1.42	<2.09
1,2,3,7,8-PeCDD	11.11	<13.21	27.00	<11.30	<10.39	<5.39	<8.04
1,2,3,4,7,8-HxCDD	6.37	12.04	17.25	<7.859	<8.44	<3.60	<4.85
1,2,3,6,7,8-HxCDD	30.00	86.97	110.39	48.99	22.29	21.8	37.81
1,2,3,7,8,9-HxCDD	5.42	<10.41	38.75	10.18	10.22	<5.29	<7.18
1,2,3,4,6,7,8-HpCDD	72.44	94.68	235.24	38.85	66.06	34.3	44.15
OCDD	806.80	1051.80	2029.89	646.17	404.55	279.92	307.74
2,3,7,8-TCDF	3.57	5.74	12.77	2.16	3.51	1.69	2.06
1,2,3,7,8-PeCDF	<4.377	4.51	13.55	<3.85	<5.42	2.08	2.04
2,3,4,7,8-PeCDF	15.74	22.91	40.69	12.46	17.05	9.88	16.33
1,2,3,4,7,8-HxCDF	16.54	16.37	34.55	13.07	12.34	6.12	8.56
1,2,3,6,7,8-HxCDF	14.80	12.95	34.24	8.56	13.67	7.15	8.51
2,3,4,6,7,8-HxCDF	12.61	10.25	27.34	<9.46	<8.60	<5.31	<5.39
1,2,3,7,8,9-HxCDF	<9.854	<13.15	<23.68	<14.87	<12.14	<5.21	<7.34
1,2,3,4,6,7,8-HpCDF	53.50	41.13	134.18	22.04	44.73	13.37	9.65
1,2,3,4,7,8,9-HpCDF	9.02	<10.96	<20.02	<11.4	<14.47	<3.45	<8.38
OCDF	32.05	28.43	164.79	22.59	33.24	14.35	10.09
105#PCB	398.40	1472.43	1613.17	427.45	9244.46	902.54	1190.47
114#PCB	113.52	301.59	249.00	135.43	1906.43	384.99	347.14
118#PCB	1829.45	6221.92	8074.46	2527.17	43941.44	5000.72	6600.25
123#PCB	29.43	47.53	408.37	24.94	1849.15	110.55	193.00
156#PCB	1499.89	3201.44	2207.09	1390.38	13916.56	2960.17	3001.67
157#PCB	306.42	605.98	469.40	286.20	2772.82	587.76	687.94
167#PCB	381.38	1065.98	1202.07	621.17	6479.97	1160.89	1396.43
189#PCB	263.21	604.86	208.14	83.83	1241.07	707.88	628.17
77#PCB	75.01	139.55	207.85	45.73	209.28	49.69	67.59
81#PCB	9.18	19.15	<31.34	<4.51	13.03	4.83	7.21
126#PCB	74.00	213.04	224.94	42.96	192.01	85.29	155.44
169#PCB	66.08	93.92	<72.89	45.39	74.29	57.74	87.20
conc. (ng g^{-1} f.w.)							
PCB-28	2.27	11.53	135.16	2.90	7.85	6.26	18.00
PCB-52	2.31	6.72	13.93	3.32	8.42	2.2	7.20
PCB-101	3.18	12.32	21.08	4.21	12.67	4.92	13.26
PCB-153	22.13	270.50	198.19	112.31	233.53	22.86	15.04
PCB-138	16.13	181.63	152.68	80.21	163.97	26.85	64.04
PCB-180	18.13	232.42	123.10	75.53	121.08	27.19	65.29
pg WHO-TEQ g^{-1} f.w.							
PCDDs/Fs	33.41	46.96	88.58	32.58	33.42	17.49	27.24
DL-PCBs	9.29	25.16	25.75	5.97	34.96	11.76	19.32
TOTAL-PCB BCR (ng g^{-1} f.w.)	64.15	715.12	644.14	278.48	547.52	90.28	182.83

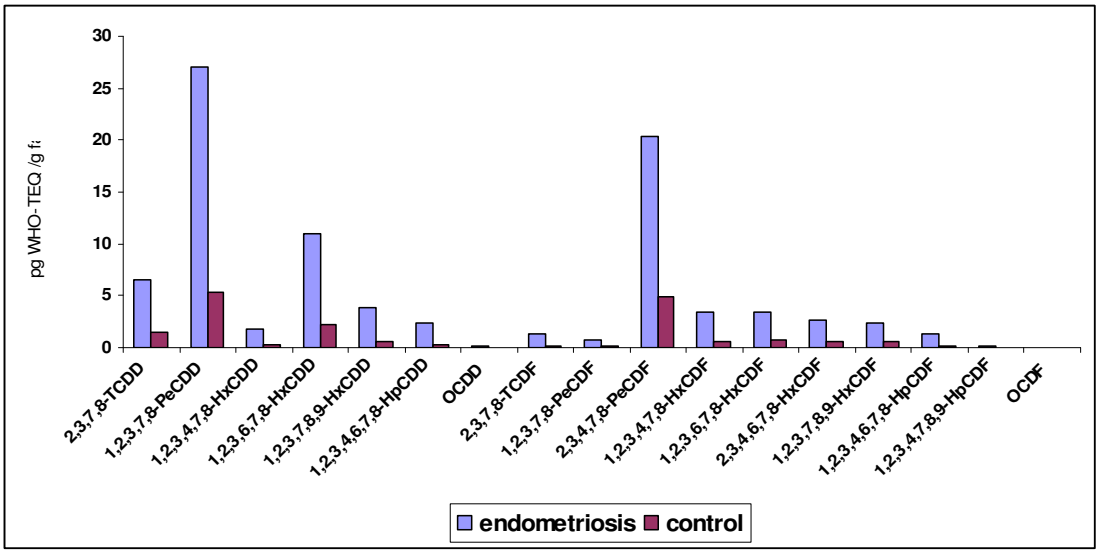

Figure 1. PCDD/F toxic levels congener distribution in serum from subjects with sever deep endometriosis and serum samples from control subjects.