Adipose tissue concentrations of PCB, HCB, Chlordane, PBDE and P,P´-DDE and the risk for endometrial cancer

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Introduction

Environmental pollutants with hormonal activity, such as xenoestrogens, have for several years been of concern as potential risk factors for hormone dependant tumors[1]. Impacts of increasing levels of xenoestrogens have been observed in aquatic organisms.[2] In humans concern has been focused on "endocrine disrupting chemicals" with either estrogenic or antiestrogenic activities. Some persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), and especially the hydroxylated metabolites, and chlordanes, have been postulated to be endocrine disruptors.[3, 4] PCBs have been shown to reverse gonadal sex in turtle[5] and abnormalities of reproductive development has been described in juvenile alligators living in contaminated environment in Florida.[6, 7] Hexachlorobenzene (HCB) has been shown to have endocrine-disrupting properties.[8] Also p,p'-dichlorodiphenyl-dichloroethylene (*p,p*'-DDE) the most persistent metabolite of p,p´-DDT has been postulated to be an environmental endocrine disruptor.[3]

In a case-control study on patients with testicular cancer we found higher concentrations of PCBs, HCB and chlordanes in mothers to cases than in mothers to controls. Similar concentrations were found in cases with testicular cancer as in the population controls.[9] The study gave support to the hypothesis that exposure to endocrine disruptors during the fetal period may be of etiologic importance in the etiology of testicular cancer. Another hormone dependent cancer is endometrial cancer. It accounted for 5.8 % of all cancers incidents among Swedish women in 2002.[10] The cumulative probability of developing the disease before 85 years of age was 2.8% in 2002. Estrogen replacement has been suggested as a risk factor among several others. The first cases of endometrial cancer among women using estrogen replacement therapy were reported in early 1960's.[11]

Is there a relationship between levels of POPs and incidence rate? The aim is to investigate adipose tissue concentrations of certain POPs, parity and hormone replacement as potential risk factors in women in relation to development of endometrial cancer.

Material and Methods

Women undergoing hysterectomy either for cancer or a benign disease at the county hospital in Karlstad and at the university hospital, in Örebro Central Sweden, were recruited for the study during 1997-1998. Approximately 5 g adipose tissue was removed for the chemical analysis of POPs. Of the number of samples (115 out of 137) included in the study, 66 cases and 34 controls were recruited from the hospital in Karlstad and 10 cases and 5 controls form the hospital in Örebro. The samples were coded so that individual data were not disclosed. They were stored in a freezer at -20^{o} C until analysis. We asked all participating subjects to answer a questionnaire about several reproductive factors, hormone use, and length and body weights. Any change of weight during the last year was recorded. Body Mass Index (BMI) was calculated for all subjects.

Chemical Analysis

The adipose tissue samples were analyzed as coded samples. We determined 37 congeners of the most abundant polychlorinated biphenyls (PCBs) in human tissue. The sum of these 37 congeners is presented here in addition to *p,p'*-DDE, HCB, six chlordane congeners and the sum of 10 congeners of polybrominated diphenylethers (PBDEs). These were TriBDE #28, TetraBDE #47, #66, PentatBDE #100, #99, #85, HexaBDE #154, #153, #138 and HeptaBDE # 183. The adipose tissue samples were homogenized with Na_2SO_4 and fortified with ^{13}C-labelled internal standards. The compounds were extracted from the tissue homogenates by supercritical fluid extraction (SFE) using CO_2 as extraction media, and potential interferences were removed by silica chromatography. The lipid content for each sample was determined gravimetrically in a sub sample. Congener specific analyses and quantification of the organochlorines and the brominated diphenyl ethers was done by high-resolution gas chromatography and mass spectrometry, HRGC-MS running in SIM-mode, by EI and NCI (the last for the brominated compounds). We measured the two most abundant ions of the chlorine cluster of the molecular ion for each compound in addition to the one ion for the 12 ^{13}C labelled internal standards (IS) and the 3 recovery standards (RS). For the brominated compounds we monitored masses 69 and 71. A quantification mixture including all compounds in addition to the IS and RS was used to calculate relative response factors (RRF). These RRFs were used to calculate the compound levels in the samples. In addition, we calculated the recovery of the IS. All recoveries of the 12 different IS were between 50-120%. We processed one laboratory blank sample with each set of 9 samples. All blank levels were < 10% of the levels reported for any of the compounds. The method detection level (MDL) defined with a S/N ratio > 3 was in the range of 0.3-1 ng/g depending on the compound and the sample size. All results are expressed in ng/g lipid. We assured external QA/QC by successfully participating in international round robin or intercalibration studies organized by both AMAP (Arctic Monitoring Assessment Program) and IUPAC (International Union of Pure and Applied Chemistry). When, in a few samples, some of the PCB-congeners were under the detection limit (nd) 50% of the detection limit was used in the calculations.

Statistical Analysis

We performed unconditional logistic regression analysis using the Stata program (Stata/SE 8.2 for Windows; StataCorp, College Station, TX) for calculation of odds ratio (OR) and 95% confidence interval (CI). In the analyses adjustment was made for age and Body Mass Index (BMI) at the time of sampling. The median concentration in the controls was used as cut-off value in the calculations of ORs and CIs since no biological relevant cut-off exists. OR and 95% CI for organohalogen compounds was calculated in relation to certain covariates such as breast-feeding, number of children and hormone replacement therapy.

Results and Discussion

The final study consisted of 76 cases and 39 controls. We present adipose tissue concentrations of organohalogen compounds in Table 1. Higher concentrations of the studied chemicals were found in the cases, but this was partly explained by older age in the cases (mean age 68) than in the controls (mean age 61). In the statistics we adjusted for age.

Table 1. Adipose tissue concentrations of organohalogens compounds (ng/g lipid) in 76 cases with endometrial cancer and 39 controls.

POP	Number	Mean	Median	Min	Max
Sum of PCBs					
— cases	76	699	599	218	3285
— controls	39	611	545	247	1649
HCB					
— cases	76	43.5	38.1	12.9	148
— controls	39	34.2	29.4	10.1	77.8
p,p'-DDE					
— cases	76	517	418	4.0	1767
— controls	39	393	256	43.4	1296
Sum of chlordanes					
— cases	75	71.4	57.7	22.4	208.3
— contorols	39	57.7	53.5	12.7	139
Sum of PBDEs					
— cases	75	2.17	1.48	0.21	22.7
— controls	39	1.70	1.24	0.40	6.31

In Table 2 are included ORs and 95% CIs for the different compounds. ORs were close to unity. Regarding *p,p´*-DDE we calculated OR = 1.9 and 95% CI = 0.8-4.8 The PCB congeners were further analysed according to structural and biological activity. No association was found.

Table 2. Odds ratio (OR) and 95% confidence interval (CI) for cases with endometrial cancer, all types combined. The median concentration of the chemicals in the controls was used as cut-off value. Numbers > median (expressed in ng/g lipid) are shown for cases and controls. Adjustment was made for age and BMI.

POP	Cases/controls	OR	95 % CI
Sum of PCBs	46/19	0.9	0.4 – 2.3
HCB	47/19	0.8	0.3 – 2.1
p,p-DDE	55/19	1.9	0.8 – 4.8
-Low	32/10	2.4	0.8 – 6.8
-High	23/9	1.3	0.4 – 4.1
Sum of chlordanes	45/19	0.7	0.2 – 1.8
Sum of PBDEs	44/19	1.5	0.6 – 3.4

We present in Table 3 results of analyses of adipose tissue concentrations of *p,p´*-DDE and estrogen replacement . Regarding ever use of estrogen replacement hormones the highest OR was found in the group with *p,p´*-DDE concentration > median in the controls, OR = 2.3, 95% CI = 0.6-8.6.

Table 3. Odds radio (OR) and 95% confidence interval (95% CI) for *p,p´*-DDE and parity and hormone replacement.

Parity and hormone replacement	OR	95% CI
Number of livebirth		
-0-1 child	1.9	0.4-9.3
-> 1 child	1.9	0.6-6.0
Estrogen (ER) replacement, ever		
-ER never, *p,p´*-DDE ≤ median	(1.0)	-
-ER ever, *p,p´*-DDE ≤ median	0.8	0.2-3.3
-ER never, *p,p´*-DDE >median	1.6	0.5-4.8
-ER ever, *p,p´*-DDE >median	2.3	0.6-8.6
Estrogen (ER) replacement, ever, adenocarcinoma only		
-ER never, *p,p´*-DDE ≤ median	(1.0)	-
-ER ever, *p,p´*-DDE ≤ median	0.7	0.2-3.1
-ER never, *p,p´*-DDE >median	1.5	0.5-4.8
-ER ever, *p,p´*-DDE >median	2.5	0.7-9.8

In this study patients who underwent hysterectomy were asked to participate. This was done in a consecutive manner by the surgeon involved in the study. Since no selection of the patients regarding surgeon occurred no selection bias was introduced. All patients agreed to participate. During the surgery approximately 5 g adipose tissue was removed from the abdominal wall for the chemical analysis.

Body mass index might influence the concentration of organohalogen compounds. Furthermore, the concentrations increase with age. All results were adjusted for BMI and age, c.f. our results on non-Hodgkin lympoma.[12] No subject reported occupational exposure to the studied chemicals.

The studied organochlorines are lipophilic chemicals that bioaccumulate in human body. Regarding PCB half-life has been estimated to be between 7-30 years measured in the human serum.[13] For p,p'-DDE the half-life in plasma is reported approximately 10 years[16] and for chlordanes 10-20 years.[17]

PCBs, and especially the hydroxylated metabolites, and chlordanes, have been postulated to be endocrine disruptors.[2-4, 16] PCBs have been shown to reverse gonadal sex in turtle[5] and abnormalities of reproductive development has been described in juvenile alligators living in contaminated environment in Florida. [6, 7] HCB has been shown to have endocrine-disrupting properties.[8] Also p,p'-DDE has been postulated to be an environmental endocrine disruptor.[3]

In this study increased OR was found for *p,p´*-DDE in most analyses. The risk was highest in cases that reported ever use of estrogen replacement therapy. This might be of interest since such treatment has been associated with an increased risk for endometrial cancer in an earlier meta analysis. Thus, an interaction with *p,p´*-DDE is indicated in this study.

Conclusion

Possible risk of endometrial cancer associated with environmental endocrine disruptors was evaluated in this case-control study. We analysed the adipose tissue concentrations of polychlorinated biphenyls, hexachlorobenzene, *p,p´*-DDE, chlordanes and polybrominated biphenyls in 76 cases with endometrial cancer and 39 controls with benign endometrial hyperplasia. For the different chemicals odds ratios (ORs) and 95 % confidence intervals (CI) were close to unity taking the median concentration among the controls as cut off value. However, for *p,p´*-DDE OR = 1.9, 95 % CI = 0.8-4.8 was obtained. Additional estrogen replacement therapy yielded in this category OR = 2.3, 95 % CI = 0.6-6.8. The results indicate an interaction between *p,p´*-DDE and estrogen replacement drugs in the aetiology of endometrial cancer. Future studies should assess concentrations of POPs and their potential interactions with endogenous and exogenous hormones.

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