DECREASED SURVIVAL IN PATIENTS WITH PANCREATIC CANCER ASSOCIATED WITH CONCENTRATIONS OF ORGANOCHLORINES IN ADIPOSE TISSUE

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Abstract
Adipose tissue concentrations of polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs), 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene (DDE), hexachlorobenzene (HCB) and chlordanes in 21 cases with exocrine pancreatic cancer and 59 controls were analysed. Significantly increased concentrations of PCBs, HCB and sum of chlordanes were found in the cases. For PCBs, odds ratio (OR) could not be calculated since all cases showed larger concentrations than the median in the controls. HCB yielded an OR of 53.0 with a 95 % confidence interval (CI) = 4.64-605. The sum of chlordanes showed an OR of 18.4 with a 95 % CI = 2.71-124. Body mass index (BMI) at the time of tissue sampling was significantly lower for the cases. This might have influenced the results. However, using the BMI from one year earlier or readjusting the concentrations of POPs to the same percentage as weight loss did not change the results. Survival of the cases was shorter in the group with the concentration of POPs larger than the median among cases, significantly for sum of PCBs (147 vs. 294 days), *p,p'*-DDE (134 vs. 302), and sum of chlordanes (142 vs. 294). All results were based on low number of cases and must be interpreted with caution.

Introduction

The etiology of pancreatic cancer is poorly understood. Smoking is the only established risk factor with about 2-fold increased risk.[1] In 2004 the number of incident cases in Sweden was 908 in a population of 9 million inhabitants. It constituted 1.6 % of male and 2.0 % of female cancer. Pancreatic cancer has been related to organochlorine compounds,[2] possibly through modulation of K-*ras* activity.[3] Occupational exposure to pesticides,[4] chemicals,[5] and especially mineral oils in transformer manufacture,[6] has been described as a risk factor for pancreatic cancer. The aim of this study was to determine concentrations of a large number of POPs in patients with pancreatic cancer and comparable controls. Both cases and controls were inhabitants in the same geographical area in mid-Sweden.

Materials and Methods

In total 21 patients were recruited for the study. All diagnoses were histopathological confirmed to be exocrine pancreatic adenocarcinoma. About 10-gram adipose tissue was taken from the abdominal wall either in local anaesthesia or during surgery for pancreatic cancer. No patient had received chemotherapy or radiotherapy before sampling. As controls 59 persons, included in two similar studies performed during the same time period, were used. Both cases and controls were asked to answer a questionnaire on e.g. previous occupations, chemical exposures, smoking habits, length, current weight as well as the weight both one and 10 years earlier. All studies were approved by the local ethical committee.

Chemical analysis

All samples were given a unique id-code that did not show if it was a case or a control during the chemical analysis. Approximately 1 g of the samples were homogenised with Na_2SO_4 and fortified with ^{13}C-labelled internal PCB and PBDE standards. The compounds of interest were extracted from the tissue homogenates by supercritical fluid extraction (SFE) using CO_2 as extraction media.[7] In addition, one laboratory blank sample and one reference sample of each set of 8 samples were analysed. The lipid content of each sample was determined gravimetrically from a sub-sample. Congener specific analysis and quantification of PCBs, PBDEs, chlordanes, DDE and HCB was performed by gas chromatography-mass spectrometry (GC-MS), running in selected ion monitoring (SIM) mode, using electron impact (EI) or negative chemical ionization (NCI). The two most abundant ions of the chlorine cluster of the molecular ion for each compound were measured, as well as one ion for the ^{13}C labelled internal standards (IS) and recovery standards (RS). A quantification standard 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. The recovery of the internal standard was calculated as well. All recoveries were between 50-120% and all blank levels were < 10% of the levels reported for all compounds. The levels of detection, defined with a signal to noise (S/N) ratio > 3, were 0.3-1 ng/g depending on the compound and the amount of sample.

Statistical Methods

Unconditional logistic regression analysis was performed 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). Adjustment was made for sex, 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. The Stata program was also used for descriptive statistics and Wilcoxon rank sum tests for calculation of *p*-values. The material was further adjusted for BMI one and ten years before tissue sampling. POPs are concentrated in adipose tissue and weight loss might increase the adipose tissue concentration.[8] Statistical analysis was performed also on adjusted tissue concentrations. These concentrations were calculated taking the weight loss during the last 10 years into account.

Results and Discussion

Concentrations of POPs in cases and controls are displayed in Table 1. Significantly higher concentrations of the sum of PCBs, congener PCB #153, HCB, sum of chlordanes and sum of PBDEs were found in the cases. For *pp*'-DDE no significant difference was seen (p=0.21). OR and 95 % CI for the different POPs are shown in Table 2. For the sum of PCBs and PCB #153 no OR could be calculated since the concentration in all cases was larger than the median concentration in controls. Significantly increased ORs were found for HCB and chlordanes whereas for *pp*'-DDE OR = 2.39, 95 % CI = 0.73-7.78 and for sum of PBDEs OR = 3.90, 95 % CI = 0.93-16.3 were calculated.

Table 1. Adipose tissue concentrations (ng/g lipid) in cases and controls.

	Number	Mean	Median	Min	Max	p[1]
Sum of PCBs						
— cases	21	1687	1304	802	4053	<0.0001
— controls	59	757	669	238	4087	
PCB #153						
— cases	21	474	353	206	1367	<0.0001
— controls	59	185	156	57	741	
HCB						
— cases	21	51	46	28	80	0.0002
— controls	59	44	29	10	680	
p,p'-DDE						
— cases	21	648	397	60	2827	0.21
— controls	59	438	261	41	2419	
Sum of chlordanes						
— cases	21	124	95	38	443	0.0004
— controls	59	66	56	13	192	
Sum of PBDEs						
— cases	21	5.1	3.1	1.2	33	0.0004
— controls	59	2.3	1.6	0.40	18	

Table 2. Odds ratio (OR) and 95 % confidence interval (CI) for cases with pancreatic cancer.

	Cases/Controls	OR	95 % CI
HCB	20/29	53.0	4.64 – 605
p,p'-DDE	14/29	2.39	0.73 – 7.78
Sum of chlordanes	19/29	18.4	2.71 – 124
Sum of PBDEs	18/29	3.90	0.93 – 16.3

Additional adjustment was made among the cases for BMI one year before sampling. The results were similar as the results presented in Table 2. The only significant change was seen for the sum of PBDEs that yielded OR = 7.67, 95 % CI = 1.53-38.5. Adjustment was also made for smoking and diabetes without any significant changes of the results. When calculating the concentrations in the cases one year before diagnosis assuming that the current concentration had increased with same percentage as loss of weight during one year no significant changes were found. All cases deceased during the project with a median survival of 275 days (mean = 323, range = 0-1236). The median of the survival rate was shorter for cases with the concentration of POPs larger than median concentration in all cases as shown in Fig 1. This difference was significant for sum of PCBs, *p,p'*-DDE and sum of chlordanes (Table 3). The difference for sum of PCBs was 147 days, for *p,p'*-DDE 168 days and for the sum of chlordanes 152 days shorter survival.

Fig 1. Survival in days in relation to adipose tissue concentrations of POPs. Median concentration among cases was used as cut-off value

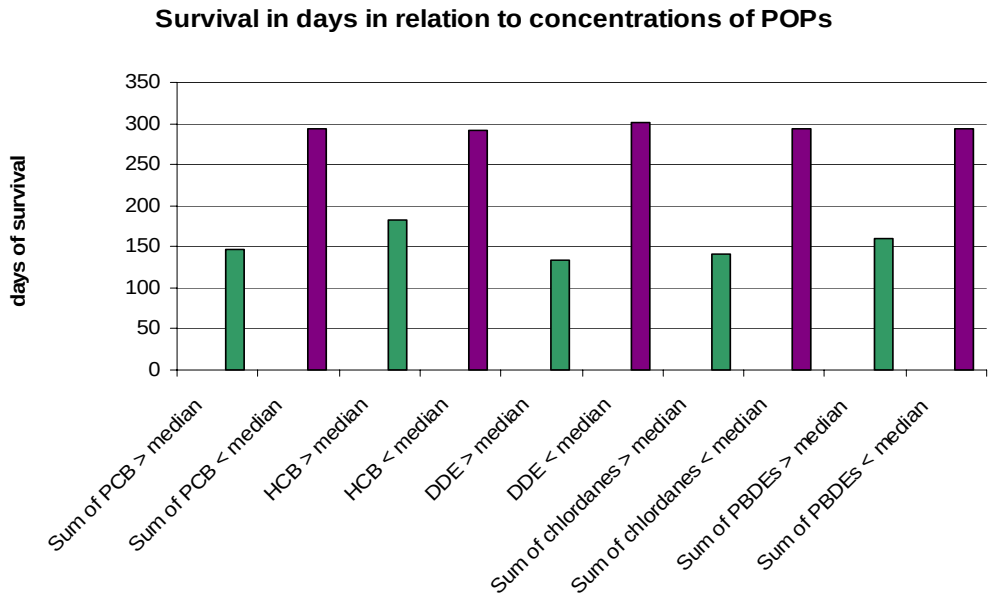

Table 3. P-values for the statistical analyse of survival in relation to concentrations of POPs.

	Number	P[1]
— sum of PCBs ≤ median	11	0.02
— sum of PCB > median	10	
— HCB ≤ median	11	0.83
— HCB > median	10	
— *p,p'*-DDE ≤ median	11	0.004
— *p,p'*-DDE > median	10	
— Sum of chlordanes ≤ median	11	0.01
— Sum of chlordanes > median	10	
— sum of PBDEs ≤ median	11	0.09
— sum of PBDEs > median	10	

This whole study was based on incident cases with newly diagnosed exocrine pancreatic cancer. No patient had received chemotherapy or radiotherapy before sampling of adipose tissue. The main result of this study was significantly increased concentrations of sum of PCBs, PCB#153, HCB, chlordanes and sum of PBDEs in the cases using Wilcoxon rank sum test for calculation of *p*-values. In the logistic regression analysis significantly increased ORs were found for the sum of PCBs, PCB #153, HCB, and chlordanes. Pancreatic cancer patients loose a significant amount of weight during the cause of their disease. Adipose tissue concentrations of POPs may increase due to decrease of weight.[8] For further statistical analysis the assumption that the concentration increased with the same percentage as weight loss during the year before tissue sampling was made. Thus, the concentrations

were recalculated for the cases. However, the results were similar to the earlier calculations and it seems reasonable that the adipose tissue concentrations were higher in the cases than in the controls in spite of weight loss. Furthermore, it is noteworthy that no significant differences were found for *p,p'*-DDE. Tissue concentration due to weight loss should have been equal for the different POPs. This finding indicates that certain POPs are increased in patients with pancreatic cancer. Our results are in accordance with previous reports.[2,3,9] Interestingly, according to the Swedish Cancer Registry the highest incidence of pancreatic cancer was seen during the 1970's and 1980's both in men and women. The incidence started to decline in the late 1980's and during 1990's and has remained stable during 2000's. This decline of the incidence occurred some years later than the decreasing concentrations of POPs, e.g., PCBs, in mother's milk in Sweden[10] and may indicate influence of environmental risk factors for pancreatic cancer. Of interest was the significantly decreased median survival for patients with pancreatic cancer with concentrations of sum of PCBs, *p,p'*-DDE and sum of chlordanes > median in all cases. To our knowledge this has not been reported before. There might be an interaction between the progress of the disease with anorexia and toxic effects from POPs in the clinical course of pancreatic cancer.

A number of industrial cohort studies have studied cancer mortality among subjects occupationally exposed to PCB. Most studies show increased cancer risks but the types of cancer vary. Mainly liver cancer, brain tumours and prostate cancer have been associated with occupational PCB exposure.[11,12,13,14] In summary this study showed increased concentrations of certain POPs in patients with pancreatic cancer and decreased survival in the patients with concentration higher than median in all cases. However, the results were based on low numbers and must be interpreted with caution. Furthermore, the wasting syndrome inherent in pancreatic cancer patients may have influenced the concentrations of POPs compared with the comparison group. In the clinical course some pancreatic cancer patients have an unexpected short survival. Further studies should explore a potential impact of POPs, if any.

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