Organochlorine Compounds (DDE and PCB) in Plasma and Breast Cyst Fluid of Women with Benign Breast Disease[1]

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Abstract

The organochlorines, dichloro-diphenyl-trichloroethane and polychlorinated biphenyl (PCB) are pervasive environmental contaminants. Results from previous studies have been conflicting regarding the relationship between the internal dose of these organochlorine residues and breast cancer risk. To determine whether these compounds are present in breast cyst fluids and whether cyst fluid and plasma concentrations are correlated, we analyzed organochlorines in paired cyst fluid and plasma samples from 24 subjects using gas chromatography and electron capture detection. All but one of the women had a history of multiple cysts, suggesting that they were at elevated risk for future breast cancer. DDE (a metabolite of dichloro-diphenyl-trichloroethane) was present in 22 of the cyst samples and PCB was detected in 19 of the cyst samples. Organochlorine levels were more concentrated in the plasma than in breast cyst fluids. Levels of DDE in plasma were significantly correlated with those in cyst fluid ($r = 0.73$; $P < 0.001$); in contrast to PCB levels in cyst and plasma ($r = 0.37$; $P = 0.12$). Congener specific analysis of the PCBs showed that some individual congeners were preferentially excluded from or concentrated in the cyst fluid. To our knowledge, this study is the first to demonstrate that PCB and DDE are present in cyst fluids and thus in contact with the ductal epithelium of the breast. These results support the use of plasma DDE as a proxy for DDE in the target tissue in research on the role of environmental factors in breast cancer.

Introduction

The organochlorines, dichloro-diphenyl-trichloroethane and PCB[3] are pervasive environmental contaminants as a result of their extensive past use as pesticides and industrial compounds. These compounds are sequestered in lipid-rich tissue where their long half-life results in their accumulation with age and allows them to persist for the lifetime of an individual (1). These compounds have varying degrees of estrogen-like properties, are proven animal carcinogens, and are suspected human carcinogens (2–4).

DDE and PCB are secreted in breast milk (5–8) and have also been identified in breast tissue (9–11). Elevated levels of DDE and PCB have been reported in the breast adipose tissue of breast cancer cases relative to matched controls (12). A 1993 study reported that the relative risk of breast cancer increased with DDE (but not PCB) plasma concentration (13); another study reported elevated risk with plasma DDE among women with estrogen receptor-positive tumors (14). A subsequent investigation did not find a significant relationship between DDE or PCB levels in plasma and breast cancer risk, although there was a positive trend for DDE among African-American and Caucasian women (15). Recently, a case-control study of 236 cases nested within the Nurses' Health Study found no association between blood levels of DDE and PCB and breast cancer (16). Similarly, a hospital based case-control study in Mexico City saw no association between breast cancer status and blood DDE levels (17). Inconsistent results may be due to varying levels of exposure or differences in the ethnic composition of the study population. The issue of whether DDE exposure is associated with breast cancer development is controversial and clearly not yet resolved.

Within the broad category of benign breast disease, gross cystic disease is a common phenomenon characterized by the presence of fluid-containing cysts. Breast cysts are fluid-filled vessels arising in terminal duct lobular units. Endogenous compounds found in cyst fluid may arise from passive or active transport of chemical constituents from the mammary epithelium into the ducts. Some, but not all, epidemiological studies have shown a modest (approximately 2-fold) increased risk of future breast cancer associated with gross cystic disease, with a history of multiple cysts conveying the greatest risk. These studies demonstrate higher risks (3–4-fold) among women who exhibit atypical hyperplastic features.

Cyst fluid can provide valuable information on the biochemical milieu of the breast. The fluid is itself a complex mixture containing a variety of endogenous and exogenous compounds whose concentrations may differ from those in the blood plasma. The distribution of many breast cyst fluid constituents (*e.g.*, DHEA-S, estrone-3-sulfate, K^+, Na^+, and EGF)

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[3] The abbreviations used are: PCB, polychlorinated biphenyl; DDE, a metabolite of dichloro-diphenyl-trichloroethane; ppb, part per billion.

has been shown to be bimodal in nature, and cysts have been categorized based on the ratio of potassium and sodium (18–21). Type I (high $K+/Na^+$ ratio) cysts are thought to be secretory in nature; in contrast to Type II cysts (low K^+/Na^+ ratio), which seem to be derived from the passive transport of plasma (18). The presence of Type I cysts has been associated with an increased risk for the development of future cysts, suggesting that the Type I cyst is a marker of active gross cystic disease (19, 20).

The aim of this exploratory study was to determine whether levels of DDE and PCB in plasma reflect cyst fluid levels in the target tissue. A finding that contaminant levels in these two compartments are associated provides assurance that blood measurements of DDE and PCB are a relevant exposure index for studies of breast cancer etiology. In this cross-sectional study, paired cyst and plasma samples from patients with gross cystic disease seen at the Columbia-Presbyterian Medical Center were analyzed for DDE and PCB levels.

Materials and Methods

Study Design. Since 1991 the Columbia-Presbyterian Medical Center Breast Service has been conducting a study of the biochemistry of gross cystic disease. This cross-sectional study has been undertaken to determine the distribution and levels of various biomarkers in cyst fluids. Women who had at least one breast cyst that was aspirated in the course of examination and treatment were invited into the study. Women who agreed to take part in the study answered a short questionnaire on known or suspected breast cancer risk factors, donated blood and cyst fluid samples, and were enrolled into the Women at Risk support group at Columbia-Presbyterian Medical Center. Cyst fluid (2–6 ml) was set aside for the study, and a 10-ml blood sample was taken in heparinized tubes. Cyst fluid samples were coded, placed on ice immediately, and were frozen at −80°C within 2 h of collection. Blood samples were fractionated, coded, and frozen within 2 h at −80°C.

Paired blood plasma and cyst fluid samples were available for organochlorine analysis from a subset (n = 24) of women enrolled in the Women at Risk program. The subjects ranged in age from 31–61 years, all had been treated with needle aspiration for gross cystic disease, and all but one patient had a history of multiple cysts. Two of the women had two cysts aspirated in the same office visit and one woman had three cysts aspirated. Each of these women gave one blood sample during the office visit. Additionally, samples from a woman who had two cysts aspirated on different days (3 months apart) were analyzed.

Laboratory Methods. Organochlorine levels in blood plasma were measured, as described previously, using gas chromatography with electron capture detection (13). Complete descriptions of the quality control procedures and results for serum have been reported previously (13, 15). In these prior published reports, the limit of detection was approximately 1 ng/ml (ppb) for DDE and 2 ng/ml for PCB in blood plasma, using the IUPAC definition of 3 × SD (22) of concentrations found in a low level serum pool (approximately 1 ppb for DDE and PCB) that had been run routinely over the course of 1 year. Similar results were obtained for the breast cyst fluid, using the same method, with a larger chromatographic column (Florisil 5 g, in the clean- up step) to accommodate the different kinds of lipid in this material. Fortified cyst fluid was analyzed to ensure adequate recoveries (>90%). The total PCB represents the sum of 13 penta- to hepta-chlorobiphenyls (as reported in Ref. 23). During the 9 months in which our present samples were analyzed, the limits of detection for PCB were 0.05–0.13 ng/ml computed as 3 × SDs of PCB congeners, the average levels of which were 0.05 ng/ml in 22 determinations of the low-level serum reference pool. In these same measurements, limits of detection for DDE and PCB were 1 ng/ml (ppb). The instrumental limit of detection for individual compounds (peak-to-noise ratio, 3:1) is approximately 0.05 ng/ml for the conditions used in this study. Actual observed concentrations were used in the statistical calculations, including those measurements near the limit of detection. This approach for epidemiological data analyses achieves a more normal distribution of values and provides a better estimate of error than using assigned categorical values for very low level results near the method's limit of detection.

Differences in lipid concentrations in plasma and cyst may confound the relationship between organochlorine compounds in these two compartments. Thirty percent of the plasma and cyst fluid samples had adequate volumes and were analyzed for total cholesterol, high density and low density lipids, and triglycerides by standard techniques. For the purposes of lipid correction, total lipid concentrations were calculated using a modified version of Akins' formula for total plasma (24, 25).

Sodium and potassium levels in the cyst fluid were measured by ion selective probe techniques. K^+/Na^+ ratios were calculated for the cyst fluids, and the cysts were grouped by cyst type using a cutoff of a K^+/Na^+ ratio >3 (18). Using this cutoff, only one cyst was classified as Type I, precluding comparisons between types.

Statistical Methods. To assess whether a relationship exists between levels of organochlorines in cyst fluid and plasma, DDE and total PCB levels were analyzed on a wet weight basis. Additionally, the subset of samples with lipid measurements was analyzed on a lipid adjusted basis. Several of the cyst fluid samples did not yield interpretable chromatographs, and missing values were assigned to these samples. Because the wet weight data were not normally distributed, the values were log e-transformed. Pearson's Correlation Coefficients were calculated for cyst and plasma levels of DDE and PCB; and least squares linear regression was used to evaluate the strength of association between the variables.

To adjust for interindividual variation in plasma and cyst fluid lipid content, DDE and PCB values were divided by estimated lipid content yielding organochlorine values expressed as ng/mg lipid. Lipid adjusted organochlorine levels were then log e-transformed to normalize the data. Pearson's Correlation Coefficients and least squares linear regression analysis were used to assess lipid-adjusted organochlorine relationships between compartments.

The possibility that PCB congeners were preferentially incorporated or excluded from the cyst fluid was evaluated. Data on the four major congeners (BZ118, BZ153, BZ138, and BZ180) were analyzed. For two cyst fluid samples, chromatograph peaks for BZ180 were not seen; a value of 0 was assigned for BZ180. For each subject, plasma:cyst PCB ratios were calculated by dividing wet weight plasma levels by wet weight cyst levels for each PCB congener and for total PCBs. To avoid nondefined BZ180 ratios for the two cyst fluid samples with no BZ180 peaks, a missing value was assigned for the ratio calculations. The ratio values were normalized by log e-transformation and paired t tests were used to compare individual congener ratios to the ratio of total congeners.

With respect to the four subjects with multiple samples, the results were not altered by use of individual cyst fluid values *versus* mean values. Thus, for each of the women who

Table 1 DDE and PCB (ng/ml) in plasma and breast cyst fluid samples

ID	Age	Cyst DDE	Plasma DDE	Cyst PCB	Plasma PCB
100	43		1.860		3.100
101		1.560	6.680	1.285	1.360
102	50	2.350	9.520	3.820	12.560
103	47	0.510	1.630	5.670	3.030
104	51	0.230	4.570	1.930	4.980
105	50	1.790	4.940		5.120
106		25.270	23.670		5.370
107	46	1.910	8.670		2.950
108	43		1.410		4.190
109	44	0.400	3.200	1.200	4.100
110	49	0.530	1.400	2.195	2.680
111	48	0.395	3.530	2.975	4.850
112	50	1.065	1.900	3.900	6.200
113	35	0.460	2.790	3.460	2.190
114	50	1.110	4.090	3.790	6.130
115	48	0.710	2.150	2.520	4.230
116	46	1.500	6.990	1.120	2.400
117	31	1.440	1.230	1.560	2.080
118	40	0.065	0.020	0.870	1.010
119	61	0.760	9.710	1.010	8.540
120	51	0.240	1.410	1.720	2.560
121	43	0.720	4.850	4.090	2.550
122	42	0.360	1.650	2.350	2.550
123	52	0.110	1.590	2.060	3.210

had multiple cysts aspirated on the same day, mean values were calculated and used in the analysis. For the woman who donated cyst fluid samples on two separate occasions, each 3 months apart, results from the first cyst fluid were used.

Results

Twenty-four subjects, from whom 25 blood samples were drawn and 29 cysts were aspirated, were selected for this study. Organochlorine results were successfully obtained from all of the blood samples, but not all of the cyst fluid samples gave readable chromatographs (see Table 1 for results). This resulted in 22 paired cyst and serum DDE measurements and 19 paired cyst and serum PCB measurements. DDE and PCB were found to be more concentrated in the plasma than in breast cyst fluids (see Table 2). The ratio of plasma:cyst DDE was higher than that of PCB. Adjustment for lipid content increased the plasma:cyst ratios.

On a plasma volume basis, levels of DDE in plasma and cyst fluid were significantly associated ($\beta = 0.65$; $r = 0.73$; $P < 0.001$, $n = 22$); but PCB concentrations were not ($\beta = 0.33$; $r = 0.37$; $P = 0.12$, $n = 19$). Among samples adjusted for lipid content, the association between DDE levels in plasma and cysts remained strong ($\beta = 1.37$; $r = 0.81$, $P = 0.02$, $n = 8$), whereas PCB levels remained unassociated ($\beta = 0.09$; $r = 0.09$, $P = 0.84$, $n = 7$).

The measure of total PCBs is the sum of 13 congeners, each of which may have different solubilities in aqueous and lipid solutions. Because these differing properties may affect transport into the cyst compartment, congener-specific data from plasma and cyst fluid were analyzed. Consistent with prior studies, high concentrations of congeners BZ118, BZ153, BZ138, and BZ180 were found in both plasma and cyst fluid (23). These congeners represented on average 74% (range, 55–85%) of the total PCBs found in plasma and 71% (range, 63–90%) of the total PCBs present in the cyst fluids in this study (see Table 3 for data on these congeners). Low concentrations of congeners BZ82/151, BZ141, and BZ170 were consistently found in plasma and cyst fluid. Although the qualitative patterns of congeners were similar, the correlations between cyst and plasma levels of individual congeners were not significant. This is consistent with the absence of an association between total PCB concentrations in plasma and cyst fluid. Plasma:cyst ratios for BZ153, BZ138, and BZ118 were calculated for all 19 subjects, and BZ180 ratios were calculated for the 17 subjects whose cyst fluid samples exhibited a BZ180 peak. As shown in Fig. 1, the ratios of plasma:cyst fluid congeners BZ153, BZ138, and BZ180 were significantly higher than the ratio for total PCBs ($P < 0.01$), whereas the ratio for congener BZ118 was significantly lower ($P < 0.001$). This suggests that, relative to other congeners, BZ118 preferentially segregates into the cyst fluid compartment.

Table 2 Mean organochlorine levels in blood plasma and breast cyst fluids

	Mean	SD	n
Cyst DDE[a]	1.98	5.24	22
Plasma DDE	4.83	5.07	22
Cyst PCB	2.5	1.32	19
Plasma PCB	4.06	2.79	19
Cyst DDE (lipid adj.)[b]	0.14	0.18	8
Plasma DDE (lipid adj.)	0.72	0.51	8
Cyst PCB (lipid adj.)	0.29	0.15	7
Plasma PCB (lipid adj.)	0.71	0.36	7

[a] ng/ml.
[b] ng/mg lipid.

Discussion

To our knowledge, this study is the first to demonstrate that PCB and DDE, carcinogenic compounds that have been suspected of influencing breast cancer risk, are present in cyst fluids and thus in contact with the ductal epithelium of the breast.

Plasma DDE levels were about 6-fold higher than those in cyst fluid, but were significantly correlated with those in cyst fluid samples, supporting blood sampling for assessing the effects of DDE on risk of breast cancer. Concentrations of PCB in plasma were roughly twice those in cyst fluid. The patterns of congeners were qualitatively similar in both samples; but the two measurements of individual congeners were not correlated. This could, in part, be due to the preferential solubility of DDE and PCB congeners in certain lipids (*e.g.*, triglycerides). Cyst fluid has a higher concentration of cholesterol and a lower concentration of triglycerides than blood plasma, which probably affects the solubility of specific organochlorine compounds (26). The relatively higher concentration of BZ118 in cyst fluid than in serum may be due to the greater polarity of BZ118 relative to most of the other congeners.

The DDE and PCB levels reported here are lower than levels found in a prior study of women from the New York metropolitan area (DDE in healthy controls: 7.7 ± 6.8 ng/ml; PCB in healthy controls: 6.7 ± 2.9 ng/ml) and lower than those found in a California study (13, 15). The correlation results for DDE are consistent with those found in recent studies that saw a significant correlation between DDE levels in plasma and adipose tissue ($r = 0.867$, $P = <.001$; Refs. 27 and 28). However, in contrast to the present study, a significant correlation between plasma and adipose tissue concentrations was also found for total PCBs (27).

The regression and correlation results indicate that plasma DDE is a reasonable proxy for DDE in cyst fluid in the target

Table 3 PCB congener levels in cyst fluid and blood plasma samples (ng/ml)

ID	BZ 118		BZ 153		BZ 138		BZ 180	
	Cyst	Plasma	Cyst	Plasma	Cyst	Plasma	Cyst	Plasma
101	0.420	0.350	0.250	0.210	0.180	0.230	0.000	0.150
102	1.020	3.210	0.940	3.060	0.780	2.340	0.000	0.940
103	1.640	0.770	1.150	0.520	0.780	0.610	0.290	0.280
104	0.520	1.080	0.330	1.140	0.340	1.030	0.120	0.540
109	0.247	0.830	0.237	0.960	0.157	0.730	0.097	0.450
110	0.420	0.200	0.390	0.640	0.400	0.610	0.185	0.290
111	0.708	1.050	0.655	1.060	0.451	0.930	0.302	0.450
112	0.820	1.450	0.700	1.290	0.590	1.050	0.360	0.630
113	0.970	0.530	0.660	0.520	0.670	0.430	0.090	0.210
114	0.870	1.590	0.680	1.310	0.540	1.230	0.300	0.490
115	0.650	0.700	0.490	1.220	0.370	0.790	0.320	0.770
116	0.390	0.710	0.200	0.550	0.220	0.490	0.020	0.250
117	0.490	0.480	0.380	0.560	0.390	0.440	0.140	0.200
118	0.190	0.110	0.200	0.190	0.130	0.110	0.039	0.150
119	0.360	2.320	0.200	2.040	0.190	1.670	0.090	0.910
120	0.480	0.300	0.350	0.700	0.290	0.540	0.130	0.450
121	1.090	0.370	0.770	0.590	0.710	0.530	0.200	0.240
122	0.420	0.290	0.540	0.910	0.400	0.640	0.230	0.340
123	0.550	0.590	0.460	0.880	0.280	0.600	0.130	0.510

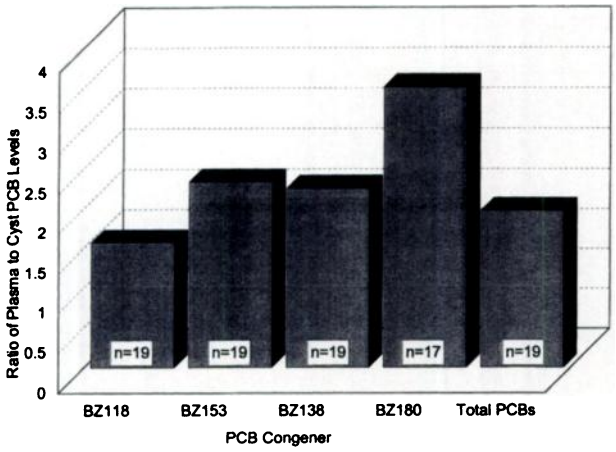

Fig. 1. The plasma:cyst ratio of PCB congeners BZ118, BZ153, BZ138, and BZ180, and total PCBs. All congener ratios are significantly different from total PCBs ($P < 0.01$).

organ, but that analysis of total PCBs in plasma may not be indicative of the levels of either total PCBs or of individual congeners in the cyst fluid. Therefore, it may be expected that other xenobiotics are preferentially transported to serum or plasma based on polarity and/or solubility.

Among the limitations of the present study is the fact that only one of the cysts sampled fell into the Type I category proposed by Angeli *et al.* (18). Even using other lower K^+/Na^+ ratios that have been proposed as cutoff points, only one or two samples could be classified as Type I (19–21). Follow-up studies are needed to evaluate a possible relationship between organochlorine levels in breast cyst fluids and cyst type as defined by K^+/Na^+ ratio. Another limitation is that due to the small volumes of cyst fluid, data on lipid content from the parent study were only available for eight of the plasma cyst fluid pairs included in the organochlorine study. This limited our ability to control for variations in lipid content. However, the wet weight results and the sub-set lipid adjusted results were correlated and any bias in the wet weight results caused by lipid variability was not systematic, and thus would be a bias toward the null.

Breast cysts, and particularly a history of multiple cysts, have been shown in some studies to confer an elevated risk of breast cancer (19, 29, 30). Thus, the women in the study can be considered a higher risk group because all but one had a history of multiple cysts. These results support the use of bloodborne DDE as an internal dosimeter in research on the role of environmental factors in breast cancer.

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