A preliminary investigation of chlorinated hydrocarbons and chronic fatigue syndrome

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Abstract

Objective: **To determine whether serum levels of chlorinated hydrocarbons are elevated in patients with chronic fatigue syndrome.**

Methods: **Chlorinated hydrocarbon levels were measured in 22 patients with chronic fatigue syndrome (CFS) (as defined by the Centers for Disease Control [CDC]); in 17 patients with CFS symptoms whose history of exposure to toxic chemicals excluded them from the research definition of CFS; and in 34 non-CFS control subjects matched for age and sex.**

Results: **DDE (1,1-dichloro-2,2-*bis* (*p*-chlorophenyl) ethene) was detected in all serum samples at levels over 0.4 ppb. The incidence of hexachlorobenzene (HCB) contamination (> 2.0 ppb) was 45% in the CFS group, compared with 21% in the non-CFS control group ($P < 0.05$). The CFS group had a significantly higher total organochlorine level (15.9 ppb; SEM, 4.4) than the control group (6.3 ppb; SEM, 1.1; $P < 0.05$). The toxic exposure group also had a higher mean organochlorine level (13.6 ppb; SEM, 6.2) than the control group, but the difference was not statistically significant. DDE and HCB comprised more than 90% of the total organochlorines measured in each of the groups.**

Conclusion: **The results suggest that recalcitrant organochlorines may have an aetiological role in CFS. There were no significant differences in serum organochlorine concentrations between CFS patients and chronic fatigue patients with a history of toxic chemical exposure. Therefore, exclusion of patients from the CDC research definition of CFS on the basis of a reported history of known exposure to toxic chemicals is not valid. The role of low-level organochlorine bioaccumulation in the development of CFS symptoms requires further investigation.**

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Chronic fatigue syndrome (CFS) is a disorder characterised by chronic fatigue and somatic symptoms. As there are no objective diagnostic tests available, the definition contains many exclusionary criteria.[1] One is exposure to toxic chemicals. Documented case histories of toxic chemical exposures and multiple chemical sensitivity indicate that these disorders can produce symptoms similar to those observed in patients with chronic fatigue syndrome.[2,3] The causes, diagnosis and treatment of all of these conditions remain controversial,[4] and there are no objective laboratory-based methods for differentiating between them.

The exclusion criteria for exposure to toxic chemicals depend on self-reported information from patients; those who may have unknowingly been exposed to toxic agents are not excluded. As solvents and organophosphate pesticides are rapidly excreted by the body, serum levels can only be validly estimated immediately after exposure.[2] On the other hand, persistent organochlorine molecules, such as hexachlorobenzene (HCB) and dichlorodiphenyltrichloroethane (DDT, or its metabolic product 1,1-dichloro-2,2-*bis* (*p*-chlorophenyl) ethene, DDE), can be measured in serum or biopsy samples. DDT and HCB are chlorinated hydrocarbon pesticides, which are an extremely stable group of lipophilic compounds that have been used extensively around the world for pest control since the early 1940s.[2] They do not readily degrade in the environment and are bioaccumulated through the food chain. Chlorinated hydrocarbons can be absorbed into the body via a number of routes, including ingestion, dermal absorption and inhalation.[5] Being lipophilic they accumulate in cell membranes, where they can alter membrane integrity and inhibit functional membrane-bound proteins.[6,7] They also accumulate in high levels in adipose tissues[8,9] and can cross the blood–brain barrier and affect neurological activity.[2]

We report a study of organochlorine levels in serum from patients with chronic fatigue syndrome (as defined by the 1988 Centers for Disease Control [CDC] criteria),[1] patients with chronic fatigue with a reported history of exposure to toxic chemicals, and healthy control subjects. We aimed to determine whether recalcitrant organochlorine levels were elevated in patients with CFS compared with healthy controls, and whether a reported history of toxic chemical exposure is a valid assessment of bioaccumulation of pesticides.

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Methods

Subjects

The patients, whose ages ranged from 10 to 67 years, were examined and diagnosed by two clinicians from August 1992 to February 1994. CFS patients (CFS group) met CDC research criteria for CFS.[1] Non-CFS subjects (control group) were recruited from relatives of the CFS patients and from unrelated subjects. Patients who met the CDC criteria except for having known exposure to toxic chemicals were studied as a toxic chemical exposure group (toxic exposure). The age and sex characteristics of the study groups are summarised in Table 1. There were no significant differences ($P>0.05$) in age or sex ratios between the groups. All subjects gave their informed consent and the project was approved by the Human Research Ethics Committee, The University of Newcastle.

Table 1: Age and sex characteristics of subjects

Group	Mean age (years, SD)	Women
Control ($n = 34$)	36.7, 8.9	56%
Chronic fatigue syndrome ($n = 22$)	40.7, 8.8	73%
Toxic exposure ($n = 17$)	43.6, 16.1	47%

Serum extraction

Whole blood (8–10 mL) was collected from fasted patients and controls (12 hours) by venepuncture into a red-top glass vacutainer (Becton Dickinson, Sydney) at either the Environmental Medical Centre or The University of Newcastle. The samples were chilled to 4°C, transported to the laboratory and the serum sample was prepared by centrifugation at 2000 rpm for 25 minutes within 24 hours. The serum (2 mL) was immediately transferred to an acid-washed 12 mL glass extraction tube and the organochlorines were extracted from the serum samples by an adaptation of the methods and recommendations of Burse et al.[10] Each extracted sample was allowed to equilibrate for one hour within a water-bath (28°C) before analysis in a gas chromatograph with electron capture detection. For each batch of samples (<10 samples per batch) an additional serum sample was spiked with a measured amount (75 ng) of pesticide standard mix. At the same time, an equivalent standard (75 ng) was also prepared in a 12 mL glass extraction tube, so that percentage recoveries could be estimated for each pesticide analysis (summarised in Table 2). Serum spiking took place just before the analysis/extraction and the minimum detection limits were determined as described by Wolff et al.[11]

Analyses

Pesticide samples were analysed with a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Palo Alto, Calif., USA) fitted with a ^{63}Ni electron capture detector, a 25.0 m, 0.20 mm internal diameter HP1 fused silica capillary column (film 0.33 μm) and a split/splitless injector. Data were stored and processed on a Hewlett-Packard Series II Integrator. The gas chromatograph was run with the following conditions: injection temperature, 250°C; detector temperature, 300°C; temperature program, 150°C–300°C at 8°C per min, with a 2-minute hold at 150°C and a 15-minute hold at 300°C; column flow rate (helium), 0.47 mL/minute; and total flow rate with ECD auxiliary make-up (nitrogen), 34.7 mL/minute. The sample injection volume was 1 μL.

All samples were analysed for pesticide contamination without knowledge of the clinical history of the patients. The clinical classifications were applied to the pesticide data at the end of the two-year period (Microsoft Access database software).[12] Data analysis software (Microsoft Excel and Statsoft Statistica)[13,14] was used to analyse the data, and analysis of variance (ANOVA), Tukey HSD (honest significant difference) test and χ^2 tests were performed as indicated in the text.

Table 2: Minimum detection limits and median percentage recoveries for organochlorine pesticides extracted from serum samples

Pesticide	Minimum detection limit (ppb)	Number of recovery evaluations	Median percentage recovery
DDT	1.4	28	104
α-HCH	1.4	28	104
Methoxychlor	1.4	37	100
γ-HCH	1.4	24	97
ß-HCH	1.4	29	97
Aldrin	0.7	30	96
HCB	2.0	10	94
Heptachlor epoxide	0.4	39	90
Endrin 1	1.4	37	90
Dieldrin	0.7	28	89
DDE	0.4	30	88
Endosulfan 2	0.7	29	88
Endosulfan 1	0.4	28	86
Endrin 2	0.4	24	67
Endrin aldehyde	7.9	25	65
Endosulfan sulfate	0.7	21	55
Endrin ketone	0.7	30	46
Heptachlor	0.7	37	41
α-Chlordane	0.6	4	63
γ-Chlordane	0.5	4	66
Polychlorinated biphenyls (PCBs)	15*	5	84
DDD	0.4	26	26

*This is the value for the total PCB evaluation. It is estimated that the individual PCB congeners were detected at levels between 2 and 5 ppb. DDT = dichlorodiphenyltrichloroethane; DDE = 1,1-dichloro-2,2-*bis* (*p*-chlorophenyl) ethene; DDD = tetrachlorodiphenylethane; HCH = hexachlorocyclohexane; HCB = hexachlorobenzene.

Chemicals

All solvents used were checked for any chromatographic interferences by concentrating 10 times the amount used in the method to 1 mL and injecting 1 μL into the gas chromatograph.[15] The pesticide standards (Table 2) were obtained from Supelco Inc. (Sigma-Aldrich, Castle Hill, NSW).

Results

DDE was detected in serum at levels greater than 0.4 ppb in all the study subjects. In addition HCB and/or dieldrin and/or heptachlor epoxide were detected in the serum samples. None of the other organochlorines listed in Table 2 was detected. At least eight additional peaks were observed in the chromatograms which could not be identified by comparison with reference standards. These may represent other halogenated serum contaminants or their detoxification metabolites which co-extracted with the organochlorines.

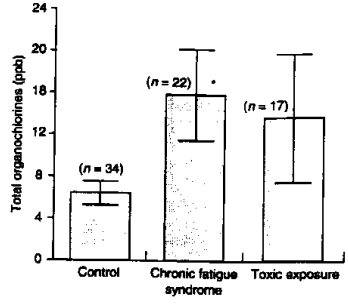
Seven of the 34 control subjects (21%) had detectable levels of HCB (i.e., at concentrations greater than 2.0 ppb), whereas 10 of the 22 CFS patients (45%; χ^2 test, $P<0.05$) and eight of the 17 subjects in the toxic exposure group (47%, not significant) had detectable levels of HCB.

The Figure shows that the mean total level of organochlorines in the CFS group was significantly higher than that in the control group ($P<0.05$), and although the value for the toxic exposure group was high compared with the control group the difference was not statistically significant. The mean levels of specific organochlorine pesticides in each clinical group are summarised in Table 3. The CFS group had a significantly higher level of DDE compared with the control group ($P<0.05$). There were no significant differences in levels of HCB or DDE between men and women in any of the groups.

Age was significantly correlated with serum concentrations of DDE measured in the control group ($r^2=0.16$; $P=0.021$) and the toxic exposure group ($r^2=0.34$; $P=0.013$), but not in the CFS group. Conversely, age was significantly correlated with serum concentrations of HCB in the CFS group ($r^2=0.54$; $P<0.001$), but not in either the control or toxic exposure groups. Heptachlor and dieldrin levels were not correlated with age in any of the groups.

Discussion

DDE, which is an end product of metabolism of DDT,[16] was present in all serum samples measured in this study, including samples from individuals who had no known history of toxic chemical exposure. While HCB, dieldrin and heptachlor epoxide were also detected in serum from subjects in groups other than the toxic exposure group, DDE and HCB were the most frequently detected and the most abundant organochlorine constituents in serum from both chronic fatigue and control subjects.



*Total organochlorine levels (± SEM) measured in the three groups. *Significant difference in levels between chronic fatigue syndrome group and control group (P < 0.05, Tukey HSD [Tukey honest significant difference test]).*

Our study clearly demonstrates that substantial levels of organochlorine pesticides occur via bioaccumulation in people with no knowledge of their toxic exposure. Thus, exclusion of patients on the basis of exposure to toxic chemicals for a research definition of CFS should include a serum or adipose tissue analysis of organochlorines, and exclusion criteria should make reference to organochlorine concentrations as well as exposure history. In view of these findings, the CDC case definition for chronic fatigue syndrome needs to be re-evaluated.

The significantly higher incidence of HCB contamination and the significantly higher concentration of DDE in CFS patients than in the control group suggest links between the development of chronic fatigue symptoms and an age-dependent bioaccumulation of organochlorine hydrocarbons. The positive correlation of age with DDE and HCB concentrations in serum is consistent with bioaccumulation of recalcitrant chemicals from the environment and/or the food chain. There was a very strong correlation of age with HCB concentration in the CFS group which was not observed in either the control or toxic exposure groups. This, together with the higher incidence of HCB in CFS subjects, suggests a specific link between HCB and chronic fatigue. It was also interesting to note that three CFS patients had organochlorine levels over 30 ppb and reported hyperallergic responses and hypersensitivity to chemicals (data not shown).

The sources of these contaminants are unknown, but could reflect occupational or environmental contamination. The detection of DDE in all subjects examined is consistent with the widespread use of DDT in Australia and the persistent nature of it and its derivatives in the environment.[2] HCB has also been used extensively in Australia as a fungicide for the protection of grain in storage.[17] Its occurrence as a contaminant in the production of other chlorinated solvents (e.g., carbon tetrachloride) and in the production of nitroso-rubber for tyres has been noted.[17] HCB and DDT are sequestered and persist in animal fats, and these animal fatty tissues comprise the major dietary source of organochlorines in Australia.[18]

Symptoms developed from prolonged low-level exposure to organochlorine pesticides may be gradual in onset and may not necessarily be associated with chemical insults. However, insecticide workers have been reported to have an elevated relative risk of mental disorders, including neurotic, depressive and sleep disorders, and an acute reaction to stress.[19] Other effects of organochlorine exposures reported in the literature (including animal and in-vitro studies)

Table 3: Organochlorine residues measured in parts per billion (SEM) in serum samples

Group	HCB (hexachlorobenzene)	Heptachlor epoxide	DDE (1,1-dichloro-2,2-*bis* (*p*-chlorophenyl) ethene)	Dieldrin	Total organochlorines
Control (C) ($n=34$)	1.4 (0.6)	0.24 (0.21)	4.27 (0.7)	0.43 (0.22)	6.3 (1.1)
Chronic fatigue syndrome (F) ($n=22$)	5.1 (2.0)	0.28 (0.17)	10.4 (3.0)	0.09 (0.07)	15.9 (4.4)
Toxic exposure (T) ($n=17$)	6.8 (4.9)	0.20 (0.22)	6.09 (2.4)	0.49 (0.37)	13.6 (6.2)
ANOVA	ns	ns	$P<0.05$	ns	$P<0.05$
Tukey HSD	C = F = T	C = F = T	C < F, C = T, F = T	C = F = T	C < F, C = T, F = T

ANOVA = Analysis of variance. Tukey HSD = Tukey honest significant difference test. ns = not significant.

are an elevated relative risk of breast cancer;[20] impairment of immune function;[21] development of endometriosis;[22] significant increases in chromosome aberrations;[23] decreases in male fertility and in the frequency of live births; and increases in stillbirths, neonatal deaths, and congenital defects in the offspring of pesticide-exposed males.[24]

Conclusions

The results of this preliminary investigation — that levels of recalcitrant organochlorines are higher in CFS patients compared with controls, and that serum organochlorine concentrations in CFS patients with and without a history of toxic chemical exposure are not significantly different — suggest that these chemicals may have an aetiological role in chronic fatigue syndrome, and that exclusion of patients from the CDC research definition on the basis of a reported history of exposure to toxic chemicals may not be valid. Future work should investigate the incidence and severity of chronic fatigue symptoms in relation to organochlorine content in both rural and city communities.

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