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Organochlorine chemicals and neurodegeneration among elderly subjects in Costa Rica

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ABSTRACT

Background: We previously screened 400 elderly Costa Ricans for neurodegenerative disease. Those reporting occupational pesticide exposure (18%) had an increased Parkinson's disease (PD) risk (OR 2.57, 95% CI 0.91–7.26), and worse cognition (Mini-Mental States Exam (MMSE) 24.5 versus 25.9 points, $p=0.01$). We subsequently measured long-lasting organochlorine pesticides (β-HCH, DDE, DDT, and dieldrin) in a sub-sample ($n=89$). Dieldrin and β-HCH have been linked to PD, and DDE to Alzheimer's disease.
Methods: We ran regression models for MMSE and tremor-at-rest to assess associations with pesticides in 89 subjects.
Results: The percent of β-HCH, DDE, DDT (parent compound for DDE), and dieldrin above their limit of detection (LOD) were 100%, 93%, 75%, and 57%, respectively. Tremor-at-rest was found in 21 subjects, and the mean MMSE was 25. Those who reported occupational pesticide exposure ($n=36$) had more detectable dieldrin samples ($p=0.005$), and higher mean levels of dieldrin ($p=0.01$), than those not reporting exposure. Other pesticides did not differ between those with and without self-reported occupational exposure. There was a positive but non-significant trend of higher risk for tremor-at-rest with higher dieldrin ($p=0.10$ for linear trend). Neither DDE nor DDT showed a relationship with MMSE. However, after excluding two outliers with the lowest MMSE scores, higher DDT levels showed some modest association with lower MMSE ($p=0.09$ for linear trend).
Conclusions: Our data are limited by small sample size. However, dieldrin was high in our population, has been previously linked to PD, and could be partly responsible for the excess PD risk seen in our population.

1. Introduction

Exposure to pesticides, primarily occupational exposure, has been studied in relation to Parkinson's disease (PD) in over 40 epidemiologic studies, which have been reviewed in meta-analyses by Van der Mark et al. (2012) and Allen and Levy (2013); these authors found overall relative risks of 1.62 (95% CI 1.40–1.88) and 1.42 (1.32–1.52), respectively. Two recent studies have identified specific long-lasting organochlorine pesticides (β-HCH, dieldrin) as increasing risk (Richardson et al., 2011; Weisskopf et al., 2010), while two others have identified paraquat and/or rotenone (Tanner et al., 2011; Costello et al., 2009). Recently the fungicide benomyl has been associated with PD (Fitzmaurice et al., 2013). Other recent work has identified organophosphates (OPs) (diazinon, chlorpyrifos, and parathion) as increasing PD risk when associated with a genetic variant (PON1) in the metabolism of OPs (Manthripragada et al., 2010; Lee et al., 2013). However, despite these advances, the specific pesticides implicated in PD have not been conclusively identified. A recent review (Pezzoli and Cereda 2013) concluded that "the literature supports the hypothesis that exposure to pesticides or solvents is a risk factor for PD" but noted that future "studies should also focus on specific chemical agents".

Exposure to pesticides has also been linked to Alzheimer's disease (AD), although there are less data than for PD. Zaganas et al. (2013) recently reviewed 14 studies, regarding poor cognition or clinical disease, and concluded that the link with pesticides was suggestive, particularly for worse cognition. Recently Richardson et al. (2014) reported that DDE, a metabolite of DDT, was

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significantly higher in AD patients than controls. The highest tertile of DDE levels was associated with an odds ratio of 4.18 for increased risk for AD (95% CI, 2.54–5.82; $p=0.001$). These authors noted that exposure of human neuroblastoma cells to DDT or DDE increased levels of amyloid precursor protein.

To investigate these links further we recently screened 400 elderly subjects (age >65) in Costa Rica for cognitive or motor deficits during 2011 (Steenland et al., 2013; Wesseling et al., 2013). These subjects were attending routine annual free medical exams; more than two-thirds of Costa Ricans over age 65 take advantage of these exams. Costa Rica is 41% rural. It has the highest per-capita use of pesticides in Central America, three times the average of the region (Bravo et al., 2011). Census data of 1984 indicated that 35% of the economically active population worked in agriculture, of whom virtually all were exposed to pesticides (Wesseling et al., 2001).

In our screening study, 18% reported past agricultural occupation with pesticide exposure. These pesticide-exposed subjects had an increased risk of PD (OR=2.57, 95% CI 0.91–7.26), whereas no excess risk was found for a diagnosis of AD or mild cognitive impairment (Steenland et al., 2013). Of our screened subjects, 92% failed Step 1 tests, which consisted of a 3 item-recall test and animal recognition test for cognition, and self-reported tremor/balance problems and a spiral drawing tests for motor deficits (Wesseling et al., 2013). Those who failed Step 1 tests passed on to screening Step 2, which consisted of a physician administering the Mini-mental State Exam (MMSE) for cognition and the Unified PD Rating Scale (UPDRS) for movement disorders; physicians were blinded to pesticide exposure status. Step 3 of the screening, for those who failed Step 2, consisted of an exam by a neurologist and a definitive diagnosis.

Regarding Step 2 tests (the outcomes in the present study), subjects with self-reported occupational pesticide exposure performed worse on the MMSE than those without occupational pesticide exposure (24.5 versus 25.9 points, $p=0.01$). Those who reported having used pesticides had also significantly elevated risks of abnormal scores on two UPDRS items, tremor-at-rest (OR 2.58, 1.28–5.23), and finger-tapping (OR=2.94, 95% CI 1.03–8.41). Tremor-at-rest is a cardinal sign of PD. Thirty-three (23%) of those examined by the neurologist in Step 3 of the screening were diagnosed with possible/probable PD, 3–4 times the expected based on international data; 88% of these cases had not been previously diagnosed.

To explore further these findings, we collected blood samples from a subsample of the screened subjects during January–February of the subsequent year 2012, when subjects returned for their yearly examination, to compare organochlorine residue levels between a group of subjects with past pesticide exposures and a group without such exposures and to analyze possible relationships of these residues with tremor-at-rest and the MMSE.

2. Methods

2.1. Recruitment of study population

We recruited subjects as they returned for their annual visits in the year after our initial study. Our goal was to recruit 100 subjects, approximately half of them with reported occupational exposure to pesticides. Since only 18% of the subjects had self-reported pesticide exposure, we oversampled this group. Eleven subjects were recruited who eventually could not be included in the analysis due to missing data. Of the remaining 89 subjects, 36 (40%) had reported occupational pesticide exposure and 53 (60%) had not.

Comparing 89 subjects with data on pesticide levels to those for whom we did not have pesticide levels but were part of the study population included in the original study ($n=311$), the mean age and years of education did not differ significantly.

2.2. Laboratory methods

After obtaining IRB approvals in the United States (Emory University) and Costa Rica (Universidad Nacional and Social Security of Costa Rica), we obtained consent from the recruited subjects to obtain a blood sample at the clinic. The serum fraction was separated and a 1-mL aliquot was taken for analysis. Samples were spiked with isotopically labeled p,p'–DDT and p,p'–DDE analogs, homogenized, denatured and extracted in Costa Rica using a slight modification of the method of Barr et al. (2006).

Samples were sent to Emory for analysis. The analytes were separated and analyzed using gas chromatography-tandem mass spectrometry using a modification of the method of Barr et al. (2003) adapted for a triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA). Injections of 2 μL were used. A full calibration plot, unknowns, blanks and quality control materials were included in each analytic run. Samples were quantified using isotope dilution calibration. The limits of detection (LOD) were dieldrin (0.25 ng/mL); o,p-DDT (0.05 ng/mL); p,p'–DDT (0.05 ng/mL); o,p-DDE (0.05 ng/mL); p,p'–DDE (0.05 ng/mL) and β-HCH (0.1). The LOD was much higher than expected for dieldrin likely because of the lack of an isotopically labeled internal standard.

2.3. Statistical methods

We first analyzed whether subjects who had reported pesticide exposures had more samples above the LOD than those who had not reported exposure to pesticides. We then imputed values for samples below the LOD, by estimating an underlying log-normal distribution and drawing random samples from it (Finkelstein and Verma 2001).

Two sample t-tests were used to compare the group who had reported occupational pesticide exposure to the non-exposed group for pesticide levels, after taking the natural log of the pesticide levels (including imputed values) to ensure normality. Spearman correlation coefficients were calculated between pairs of pesticides. We regressed MMSE on pesticide levels using linear regression, adjusting for gender, age, and years of school. Continuous variables were used for age and years of schooling after categorical analyses indicating monotonic trends (inverse and positive, respectively) between these variables and MMSE. We did not collect data on other potential confounders of interest, such as body mass index or pregnancy history, as our study was a screening study, not designed for detailed questionnaires, and the time available with nurses for additional questions during the initial screening step was quite limited. We conducted an analysis by quartiles for each pesticide, and a test of trend via inclusion of a single (continuous) linear term. The referent for the quartile analyses was the lowest quartile for β-HCH, DDE, and DDT. For dieldrin, which has 43% samples below the LOD, use of the lowest quartile as a referent would not be recommended as values below the LOD were randomly assigned to those below the LOD. Therefore for dieldrin we used those values which were below the LOD as the referent, and constructed quartiles above the referent. Analyses were conducted separately for each pesticide. Data were sparse and models including all pesticides together were unstable; some pesticides were moderately correlated increasing instability.

We also ran logistic models for tremor-at-rest (yes/no), which was one of the 10 tests on the UPDRS, and the one most closely linked to a subsequent diagnosis of PD in our original larger population (the relative odds of PD for those with tremor vs. those without was 3.55 (95% CI 1.62–0.7.77)). Tremor-at-rest test had values of either 0 (no tremor), or 1 (some tremor), or 2 (more pronounced tremor). As only two subjects had a value of 2, we analyzed tremor-at-rest as either 0 (75%) or 1 (scores 1 or 2 combined; 25%); 21 of 89 subjects had a positive tremor-at-rest. Logistic models were adjusted for age and gender. Age was first considered as a categorical variable (quartiles) and showed a monotonic increasing trend with tremor; hence we included age as a continuous variable in final models. Years of school were not a predictor of tremor, and were not included in final models. Tremor-at-rest was regressed on 4 pesticides individually. Again, we conducted an analysis by quartiles for each pesticide, and a test of trend via inclusion of a single linear term. The referent for the quartile analyses was again the lowest quartile for β-HCH, DDE, and DDT, while for dieldrin we used those below the LOD as the referent, and constructed quartiles above the referent.

We also compared levels of the four pesticides to levels in US population, obtained from the NHANES 2003/2004 survey http://www.n.cdc.gov/nchs/nhanes/search/nhanes03_04.aspx for approximately 1800 subjects, chosen to represent the US population. We report the 25th, 50th, and 75th percentile of the distribution of each pesticide in our population and the NHANES population, using imputed data from our population when needed (for samples below the LOD), and also report the percent of samples below the LOD in each population.

3. Results

Table 1 shows the distribution of demographic data and pesticides levels for the subjects who had self-reported occupational pesticide exposure and those who had not. Pesticide users