Exposure to CB-153 and p,p´-DDE and human sperm chromatin integrity

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Introduction

In Sweden, the consumption of fatty fish from the Baltic Sea (off the Swedish east coast) is the single most important source of exposure to persistent organochlorine pollutants (POPs). Fishermen from the east coast have averagely higher plasma levels of polychlorinated biphenyls (PCBs) and total POP derived TEQ in plasma than both west coast fishermen and men from the general population (*1*). Dichlorodiphenyl dichloroethene (p,p´-DDE), a relevant biomarker for POP is still present in relatively high serum concentrations in men consuming fish from the Baltic Sea (*2*).

Several studies have shown that POPs are capable of interfering with reproductive and endocrine function in animals (*3-5*). Human studies have shown that exposure to PCBs and polychlorinated dibenzofurans (PCDFs) has a negative effect on male reproductive function, and especially sperm motility seems vulnerable (*6-9*). However, studies relating to human sperm genetic integrity are few (*10, 11*).

The aim of the study was to investigate whether exposure to POP using 2,2´,4,4´,5,5´-hexachlorobiphenyl (CB-153) and p,p´-DDE as biomarkers, are associated with sperm chromatin integrity. In order to ensure a sufficient variation in POP exposure fishermen from both the Swedish east ("more exposed") and west coasts ("less exposed") formed the study base.

Materials and methods

Cohorts of fishermen have been established previously (*1*). From these cohorts 96 east coast and 99 west coast fishermen were recruited, mean age 47 years, range 24-67. Information on lifestyle, reproductive and occupational history was obtained through telephone interviews.

Blood and semen samples were collected at the subjects homes. The participants were asked to keep an abstinence time for 3-4 days and collect the semen sample by masturbation 30 minutes before the arrival of the mobile laboratory. Two tubes of undiluted raw semen were put into a box with dry ice and shortly thereafter transferred into a freezer at -80°C.

Sperm Chromatin Structure Analysis (SCSA) is a flow cytometry method that uses the DNA-specific fluorescent dye acridine orange (AO), and measures the susceptibility of sperm nuclear DNA to be denaturated in situ (*12*). SCSA can detect two independent sperm abnormalities

mirrored by the parameters DNA Fragmentation Index (DFI) and High DNA stainability (HDS). Due to limited amounts of semen, samples from only 176 men could be used for (SCSA).

The CB-153 and p,p´-DDE were extracted from the serum by solid phase extraction (Isolute ENV+; IST, Hengoed, UK) using on-column degradation of the lipids and analysis by gas chromatography mass spectrometry. $^{13}C_{12}$-labeled CB-153 and $^{13}C_{12}$-labeled p,p´-DDE were used as internal standards. The CB-153 and p,p´-DDE concentrations were adjusted for total serum lipid concentration determined by enzymatic methods and expressed as ng/g lipids.

Bivariate associations were evaluated by Pearson´s correlation coefficient. CB-153 and p,p´-DDE were categorized into equally large quintiles with respect to serum exposure levels. Due to skew distributions of the DFI and HDS variables, we tested whether log transformation of these variables better fulfilled the model assumptions, which were checked by means of residual analysis.

As potential confounders we initially considered age, smoking, abstinence time, body mass index (BMI) and levels of testosterone, sexual hormone binding globulin (SHBG), follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, inhibin B and the testosterone/SHBG ratio. However, there were no associations between the exposure variables and abstinence time (r_s<0.03), smokers/nonsmokers (mean difference in CB-153 and p,p´-DDE < 7%), inhibin B (r_s<0.06), LH (r_s<0.02), testosterone (r_s<0.08). Thus, these variables were excluded from further analyses. The remaining variables were included in the models, one at a time, together with the exposure variable if they showed any association (p>0.20) with DFI or HDS. In the end only age remained in the model as a potential confounder.

If the adjusted estimates differed less than 15 % from the crude estimates we only present the crude estimates.

Results

The subjects had a median CB-153 serum level of 193 ng/g lipid (range 39-1460) and median p,p´-DDE serum level of 240 ng/g lipid (range 40-2252).

When CB-153 was categorized into quintiles the lowest exposed group differed from the other four (fig.1). Therefore, we grouped the four highest exposed quintiles (≥113 ng/g lipid) and compared with the lowest. The quintile with the lowest exposure had significantly lower levels of DFI compared to the other four groups (p<0.001). This effect remained when age was included in the model (p=0.006). The highest exposed groups had 41% (95% CI 11, 78) higher DFI compared to the lowest exposed quintile.

Regarding p,p´-DDE the pattern was less clear. When age was included in the model, the lowest exposed group (<136 ng/g lipid) did not significantly differ from the four quintiles with higher exposure levels (22 %, 95% CI -4, 53).

For the variable HDS no associations were found with CB-153 or p,p´-DDE.

Discussion

The main result of the present study was a positive association between serum levels of CB-153 and DFI, indicating that POP exposure might affect sperm DNA integrity.

To the best of our knowledge there are only two previous studies regarding the association between POP exposure and sperm chromatin damage in humans. In a study carried out in India, 21 infertile men were compared to 32 men with normal semen analyses and evidence of conception. There was a significant positive association between seminal total PCB level and the percentage of single-stranded DNA in sperms (*11*). In the other study, carried out in USA, the neutral single cell micro gel electrophoresis assay (Comet assay) was used to assess DNA integrity in 212 male

partners of sub-fertile couples. The authors did not find any statistically significant associations between sperm DNA damage and serum levels of any individual PCB congeners, sum of PCB, or p,p´-DDE, leading to the conclusion that there were no strong relationships (*10*). In this American study CB-153 serum levels ranged from 9 to 421 ng/g lipid (median 44) which is much lower than in the present study.

In the present study we used CB-153 as a biomarker for PCBs exposure and found an increased DFI among men with the highest exposure levels. The possible mechanisms of reproductive toxicity of PCB are not yet understood. Nevertheless, we have to take into consideration that individual PCB congeners and their metabolites exhibit different toxicity.

One potential mechanism whereby PCBs may produce DNA damage is through hydroxylated PCB metabolites (OH-PCBs), which are found in human serum in relatively high concentrations (*2*). These metabolites can be further oxidized to form semiquinons and quinons, which are reactive eletrophiles that may induce free radical-mediate oxidative DNA damage and strand breaks (*13, 14*). If PCBs produce oxidative damage in human sperm, this may be one potential mechanism to support the hypothesis that PCB may contribute to male infertility. There are several potential mechanistic links between POP exposure and DNA damage in sperms, but experimental studies are needed to clarify the relevance of these proposed mechanisms.

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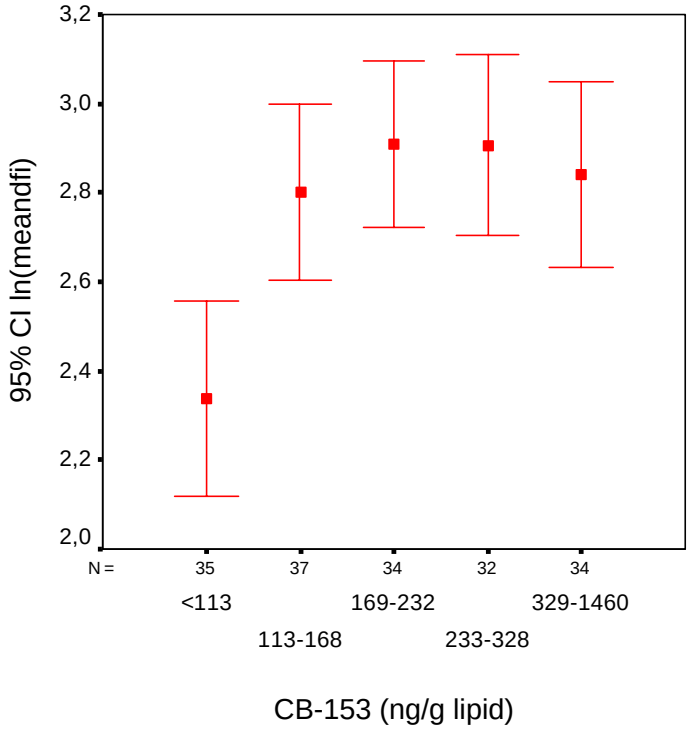

Figure 1. The association between serum concentration 2,2´4,4´5,5´-hexachlorobiphenyl (CB-153; divided into five groups) and the logarithm of DFI ($p<0.001$).

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