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Sperm chromatin structure and semen quality following occupational styrene exposure

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Occupational exposure to organic solvents may exert a detrimental effect on human spermiogenesis (1, 2). But the findings are not conclusive (3, 4). Styrene is an organic solvent used for the production of plastics. It has a well documented neurotoxic effect and may modulate dopamine metabolism in the brain and disturb the pituitary-gonadal axis. An increased proportion of sperm with abnormal morphology has been reported for workers exposed to high levels of styrene (5). Some studies of rodents have indicated decreased sperm count following styrene exposure (6), while others have not (7).

In the reinforced plastics industry, workers may be exposed to high styrene levels, while other exposures suspected to be hazardous to spermatogenesis are absent. Styrene, therefore, was selected as an organic solvent suitable for a further assessment of effects on human spermiogenesis.

In this study, 23 workers provided a semen sample at the time of being hired in the reinforced plastics industry and again after about 6 months of employment. Changes in conventional measures of semen quality and sperm susceptibility to denaturation, as assessed by the flow cytometric sperm chromatin structure assay (SCSA), were related to levels of styrene exposure.

Subjects and methods

All 131 workers hired between October 1994 and February 1995 in 4 Danish reinforced plastics companies were informed about the study on their first day at work. Altogether 37 (30%) agreed to participate and were enrolled except 3, who were ineligible because of vasectomy, previous styrene exposure, or previous welding exposure. During the following 6 months 11 of the 34

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6 The Asclepios project on occupational hazards to male reproductive capability is a biomedical research project of the European Union that was carried out in 14 European centers in 1993—1998. The project was coordinated by The Steno Institute of Public Health, University of Aarhus Denmark, and it included the following researchers: Belgium, Gent (P Kiss, A Mahmoud, M Vanhoorne, H Verstraelen); Denmark, Aarhus (A Abell, JP Bonde, SB Larsen, G Danscher, E Ernst, H Kolstad), Copenhagen (A Giwercman); England, London (A Dale, M Joffe, N Shah); Finland, Helsinki (M-L Lindbohm, H Taskinen, M Sallmen), Turku (J Lähdetie); France, Paris (P Jouannet, P Thonneau), Strasbourg (A Clavert); Germany, Erlangen (KH Schaller, W Zschiesche); Italy, Brescia (P Apostoli, S Porru), Milano (L Bisanti), Pietrasanta (L Lastrucci), Rome (M Spanò); The Netherlands, Nijmegen (N Roeleveld, H Thuis, GA Zielhuis), Zeist (W de Kort); Poland, Lodz (K Sitarek).

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workers left the companies or were, for other reasons, lost for follow-up. The 23 workers who provided 2 semen samples, 1 during the first week of employment and 1 after some 6 months of employment, were included in the analysis.

The study subjects were visited at their home address by a technician with a mobile laboratory. A semen sample was collected by masturbation and kept close to the body until examination within 1—2 hours. Semen examination was according to the present criteria of the World Health Organization (WHO). Semen volume was measured in a graded tube. Sperm density was counted twice by phase contrast microscopy in a Neubauer hemocytometer. Total sperm count was estimated by the product of sperm density and semen volume, except for 6 workers reporting spillage.

The curved line velocity of at least 100 sperms was analyzed using the Hobson sperm tracker system on a video recording obtained from an undiluted fresh semen sample in a thermostat-regulated Mackler chamber holding 37°C.

The semen smears were air dried, fixed in 96% ethanol, and stained using a modified Papanicolaou stain, and later morphology scoring was completed according to the WHO criteria by 1 laboratory technician. The eosin-Nigrosin coloring technique was used to differentiate between the vital and nonvital sperm.

Information on days of sexual abstinence before the semen sample, a period of fever within the last 3 months, and spillage during the sampling was obtained by questionnaire. The duration from provision of the sample to initial analysis was recorded.

Vulnerable chromatin structure, defined as an increased susceptibility to acid-induced denaturation in situ, was quantified by SCSA. After staining of sperm with acridine orange, the metachromatic shift from green (native DNA) to red (denatured, single-stranded DNA) fluorescence was measured by flow cytometry. The shift was expressed by αt, which is the ratio of red to total (red+green) fluorescence. After acidic denaturation, a higher proportion of single-stranded DNA is expected in structurally altered than in normally condensed chromatin. In the SCSA, αt is calculated for each sperm cell in a sample, and results are expressed as the mean (mean αt) and standard deviation of the αt distribution (SD αt). The proportion of cells outside the main population (%COMP αt) was also calculated. The SCSA followed the procedure described by Evenson (8), with minor modifications.

The postshift urinary mandelic acid concentration is a valid indicator of internal dose from occupational styrene exposure. Through 5 successive days just after hiring and through 5 additional days 6 months later, daily postshift urinary samples were collected within 1 hour after the end of the shift and kept in a refrigerator and freezer until final shipment to the University Erlangen-Nuremberg for analysis.

Mandelic acid was extracted from urine with diethylether; 3-hydroxy benzoic acid was used as the internal standard. After evaporation of the ether extract to dryness, the residue was dissolved in methanol-water (1:5), and metabolites were separated by high-pressure liquid chromatography and determined using an ultraviolet detector at 215 nm.

Altogether 187 urinary samples were collected from 28 workers out of the 34 men initially enrolled (including 8 workers who were excluded from the final analysis because they only provided 1 semen sample). Each worker was classified according to the average of all mandelic acid measurements regardless of the number of samples. The average postshift urinary mandelic acid level of the 28 workers was 65.9 mg/g creatinine. The highest exposed worker (based on 5 successive daily samples) had an average mandelic acid value of 268.3 mg/g.

Mandelic acid concentration was related to information about work conditions and work tasks obtained by questionnaire. Number of days of lamination (plastics molding) showed the strongest relation to mandelic acid level. Full-time use of a respirator or a charcoal filter mask significantly modified the internal doses. These 2 parameters explained 42% of the variability when assessed in a multivariate linear regression model. Production technology, hours of lamination per day, and type or dimensions of the product were only weak predictors of the mandelic acid level (data not shown).

For 3 of the 23 workers included in the final analysis no postshift urinary samples were available. From the questionnaire data on work conditions, they were assigned a mandelic acid value based on the results from the 28 workers with complete data.

The changes in semen parameters between the first and second samples were tested by the paired t-test. The individual changes were related to mandelic acid concentration in a linear regression analysis using the general linear modeling procedure of SAS (statistical analysis system). Because all semen parameter distributions were skewed, the parameters were transformed by the logarithm (total sperm count, proportion nonvital, all SCSA parameters), the cube root (sperm density), or the logit function (proportion with normal morphology) before the statistical testing. The mandelic acid concentration and sperm parameters (when appropriate) were treated as continuous parameters. For several potential time-dependent confounders, dummy variables for the differences within each individual between the first and second sample were generated. They were length of sexual abstinence (identical, <0 days, >0 days), fever during the 3 months before sampling (identical, 1st sample only, 2nd sample only), spillage during sampling (identical, 1st sample only, 2nd sample only), duration from provision

of the sample to examination (identical, <0 hours, >0 hours), and season (2nd sample: summer, nonsummer).

Results

Sperm density declined from a median value of 63.5 million sperm per milliliter of ejaculate to 46.0 million after 6 months of styrene exposure. The total sperm count was almost halved from an initial value of 175 million per ejaculate, while the proportion of sperm with normal morphology was reduced from the preexposure 44.3% to 38.5%. On the other hand, the proportion of nonvital sperm decreased from 14.0% to 6.5%, and the median sperm velocity increased from 66.1 μm/s to 77.8 μm/s. These changes were all statistically significant in paired t-tests.

No indications of exposure-response relationship were seen when the individual changes in semen quality were related to the postshift urinary mandelic acid concentrations, controlled for change in potential time-dependent confounders.

No changes in sperm DNA chromatin structure were detected from the first to the second sample according to the mean, the standard deviation or the %COMP of the αt distribution.

However, a weak increase in DNA-susceptibility as a function of mandelic acid concentration was indicated. But this analysis included only 14 subjects, and the trend was partially due to a decrease in the values of αt parameters in the unexposed and low-exposed workers.

Discussion

Sperm density, total sperm count, and the proportion of sperm with normal morphology declined during the styrene exposure. But these changes were not related to quantitative measures of internal styrene exposure. No indications of a detrimental effect of styrene on sperm viability or velocity were observed.

According to the scant findings of previous studies, there is little evidence that semen parameters are affected by organic solvent exposure (4). The only human data on styrene exposure originate from a cross-sectional study of workers from the reinforced plastics industry, and it showed a decrease in the proportion of sperm with normal morphology compared with that of a control group of infertility patients (5).

The changes in sperm chromatin structure parameters indicated exposure-response relationships. But the changes were in the range of the interassay variability of the SCSA as assessed in a parallel quality program.

The flow cytometric SCSA approach has demonstrated a remarkable sensitivity to detect an increased susceptibility of DNA denaturation after the exposure of rodents to a variety of environmental agents. Styrene is metabolized to styrene oxide, which is a potent mutagen, and DNA adducts have been detected in lymphocytes of styrene-exposed workers. Thus the possible association between styrene exposure and increased DNA susceptibility is biologically plausible.

Eleven plastics workers dropped out during the follow-up. They showed a median sperm density of 30 million per milliliters, which was less than half the 70 million per milliliters seen in the 23 participants. It is our impression that at least some of the dropouts participated in order to have a free infertility check up. Although we observed no indications of drop-out due to the styrene exposure level, this situation clearly illustrates the potential for selection bias in cross-sectional studies (9). To avoid selection bias on an individual level, the present analysis was therefore restricted to the 23 workers with semen samples both before and during the styrene exposure. But this restriction may have regression towards the mean as an unintended side effect, and the simple comparisons of semen parameters during exposure versus the base-line parameters must be interpreted with caution.

Semen quality is known to show seasonal changes (10). Nine of the 23 second samples collected after 6 months of exposure were collected during the summer (July-September), while all the initial samples were collected outside this period. This imbalance in the design may at least partly have attributed to the changes observed during the follow up.

The internal exposure data based on the postshift urinary mandelic acid concentration provided the possibility of exposure-response evaluation within the group of reinforced plastics workers. But the exposure gradient was modest. The postshift urinary mandelic acid concentrations of the highest exposed worker was 33% of the BEI (biological exposure index) provided by the American Conference of Governmental Industrial Hygienists.

Only 23 styrene-exposed subjects were studied. This number was sufficient to detect minor time-dependent changes of most measures in semen quality with acceptable precision. But the numbers were insufficient to elucidate fully the exposure-response relationship between mandelic acid and semen parameters. We were aware of this situation when designing the study, which was planned as a European concerted action with contributions from several centers. But only few men were recruited outside Denmark, primarily because of low worker turnover in the reinforced plastics industry.

In summary, a declining sperm count and a declining sperm proportion with normal morphology following styrene exposure is suggested. But the changes were unrelated to exposure level. An increased susceptibility of sperm DNA to in situ denaturation is also suggested, but the detected changes are within the interassay variability, and the findings must be regarded as preliminary. Any additional studies on this topic should include persons

from an industrial setting with higher styrene exposure levels and an external reference group.

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