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Sperm DNA oxidative damage and DNA adducts

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ABSTRACT

The objective of this study was to investigate DNA damage and adducts in sperm from coke oven workers who have been exposed to polycyclic aromatic hydrocarbons. A longitudinal study was conducted with repeated measurements during spermatogenesis. Coke-oven workers ($n = 112$) from a coke-oven plant served the PAH-exposed group, while administrators and security personnel ($n = 67$) served the control. Routine semen parameters (concentration, motility, vitality, and morphology) were analyzed simultaneously; the assessment of sperm DNA integrity endpoints included DNA fragmentation, bulky DNA adducts, and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dGuo). The degree of sperm DNA fragmentation was measured using the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay and sperm chromatin structure assay (SCSA). The PAH-exposed group had a significant increase in bulky DNA adducts and 8-oxo-dGuo compared to the control subjects ($Ps = 0.002$ and 0.045, respectively). Coke oven workers' percentages of DNA fragmentation and denaturation from the PAH-exposed group were not significantly different from those of the control subjects ($Ps = 0.232$ and 0.245, respectively). Routine semen parameters and DNA integrity endpoints were not correlated. Concentrations of 8-oxo-dGuo were positively correlated with percentages of DNA fragmentation measured by both TUNEL and SCSA ($Ps = 0.045$ and 0.034, respectively). However, the concentrations of 8-oxo-dGuo and percentages of DNA fragmentation did not correlate with concentrations of bulky DNA adducts. In summary, coke oven workers with chronic exposure to PAHs experienced decreased sperm DNA integrity. Oxidative stress could contribute to the degree of DNA fragmentation. Bulky DNA adducts may be independent of the formation of DNA fragmentation and oxidative adducts in sperm. Monitoring sperm DNA integrity is recommended as a part of the process of assessing the impact of occupational and environmental toxins on sperm.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are formed during the incomplete combustion of organic materials and are widely distributed into the environment in complex mixtures, such as vehicle exhaust, tobacco smoke, cooked food, and water and urban air. PAHs can undergo CYP 450 Phase I metabolism and form active intermediates, which have been reported to attach covalently to DNA in lymphocytes and tissues of organs including gonads [1]. These reactive intermediates are then capable of covalent binding to DNA, potentially initiating a carcinogenic process [2]. PAHs can form free-radical cations via the one-electron oxidation process and, thus, have the potential to cause oxidative damage to DNA [3–5]. When unrepaired, DNA adducts can cause mutations, including mutational hotspots in the p53 tumor suppressor gene and other genes, which ultimately may induce cancer [6].

Several PAH compounds have been reported to impair the reproductive capacity of males adversely, including decreased semen quality and DNA integrity [7–9]. Animal studies have reported significant levels of PAH metabolites in the testis and

Abbreviations: TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; PAHs, polycyclic aromatic hydrocarbons; 1-OHP, 1-hydroxypyrene; LC–MS/MS, liquid chromatography-mass spectrophotography/mass spectrophotography; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; SCSA, sperm chromatin structure assay; 8-oxodGuo, 8-oxo-7,8-dihydro-2′-deoxyguanosine.

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epididymis of exposed rats [10]. The substantial presence of PAH metabolites in the male gonads even after 8 h (oral), or 4 h (inhalation) post-exposure, suggests that PAHs can pass through a blood-testis barrier and then be incorporate into the Leydig lipogenic tissue [10,11]. Also, PAH exposure has been linked to oxidative DNA damage and possible single and double-DNA strand breaks [7,8]. Animal studies detected PAH-DNA adducts in testicular tissues and raised the possibility that PAHs can compromise the function of the barrier and affect spermatogenesis [12]. Limited epidemiological studies have also detected PAH-DNA adducts in human sperm [9,12,13]. Sperm DNA repair commonly occurs during the early stages of spermatogenesis (spermatocytes and early spermatids), but not in mature spermatids and spermatozoa [14]. This limited window of repair indicates the possible accumulation of non-repaired DNA damage.

Sperm quality has been used as the most convenient way to assess impact from environmental toxin exposure. However, recent reports showed semen parameters may not address the integrity of the male genome in the sperm head [15]. Common methods used to test sperm DNA fragmentation and denaturation in the clinic setting include the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay and the sperm chromatin structure assay (SCSA) [16]. Emerging evidence has suggested that sperm DNA integrity may be a better predictor of male fertility potential than routine semen parameters [17,18]. However, DNA fragmentation has been criticized for providing little specific information on the nature, mechanism, and severity of the DNA damage detected [19].

Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG), generated by the interaction of the hydroxyl radical with guanine, is one of the most abundant products of oxidative damage to DNA; it has been used as a biomarker to depict systemic oxidative stress [18,22] and to estimate oxidative stress linking occupational and environmental exposures [22,23]. The detection of a lesion has been considered important because of its abundance and mutagenic potential through the G to T transversion mutation upon replication of DNA [21]. Park et al. demonstrated that metabolic activation of PAHs by aldo-keto reductases leads to the formation of redox active o-quinones and reactive oxygen species [5], which oxidize the C-8 position of the guanine base of DNA and form 8-oxo-dG [24,25]. Recent evidence has detected 8-oxo-dGuo in human sperm [19]. However, very limited data are available to support the notion that urinary 8-oxo-dGuo could be a reliable biomarker for assessing oxidative stress and damage of sperm. Also, measurements of oxidative damage in sperm have not been fully standardized and examined due to the interference of DNA isolation from the compact nature of sperm chromatin. The use of DNA isolation and extraction methods developed and recommended by the European Committee for Standardization, could minimize oxidation during DNA extraction from tissues and cells. Such method is particularly useful for DNA extraction from sperm cells, which could facilitate the process of measuring oxidative damage to sperm more accurately and the possibility of identifying a reliable biomarker.

The objective of this study was to assess sperm DNA integrity of coke oven workers chronically exposed to PAHs as compared to the control subjects. DNA fragmentation, denaturation, 8-oxo-dGuo, and bulky adducts were assessed to determine nuclear DNA integrity of sperm. The study used well established ^{32}P-labeling for DNA adduct detection, while employing the newly established liquid chromatography-mass spectrometry/mass spectrometry with an on-line solid phase extraction procedure for 8-oxo-dGuo analysis [27,28,36].

2. Materials and methods

2. 1 Human subjects and sampling scheme

Human subjects included coke oven workers and administrative staff who worked at a steel plant in southern Taiwan. Participants were recruited during their annual health examination at the Kaohsiung Municipal Hsiao-Kang Hospital, a main municipal hospital system that provides health care for occupational workers in the southern region of Taiwan.

Coke oven workers (n = 112) served as the PAH-exposed group who had chronically exposed to PAHs, while administrative staff, including administrators and security personnel (n = 67) served as the control group with minimal exposure to PAHs. Our preliminary data showed that PAH concentrations around the coke oven processing area ranged from 15,000 ng m$^{3-1}$ to 40,000 ng m$^{3-1}$, while PAH concentrations in offices were less than 50 ng m$^{3-1}$ [29]. Criteria for human subject selection included being a male between 25 and 50 years old, having no reproductive dysfunction, and being employed at the plant more than one year. We recruited non-smokers only because tobacco smoke contains PAHs and other chemicals that could induce oxidative damage.

We repeatedly collect biological samples (Sampling I and Sampling II) throughout the entire process of spermatogenesis, which takes 74 days, including the transport on duct system. The selection of the sampling scheme is based on: (i) the regular schedule of the workers who work for 6 continuous days, followed by 2 days off; (ii) the cycle of spermatogenesis (65–75 days), which covers approximately 8 rotation cycles of coke-oven workers [8 × (2 rest days + 6 work days) = 64 days]. Furthermore, the sampling scheme won't create any significant interference with operational processes based on our discussion with the plant manager. Semen samples were collected in the evening of the 2nd rest day of 1st rotation cycle, and in the evening of the 5th and 6th work days (end-of-shift) of the 8th and 12th rotation cycles. Because PAH metabolites in urine fluctuated during the rotation cycle [29], the four urine spot samples were collected in the morning (pre-shift) and evening of the 1st work day of the 1st rotation cycle and in the morning and evening (end-of-shift) of the 6th work day of the 8th and 12th rotation cycles. One blood sample was collected in the evening of the 1st work day of the 1st rotation cycle. We collected a questionnaire from each participant in the evening of the 1 work day of the 1st rotation cycle to ascertain basic demographic data, which had the potential to covary with or to confound our main measures. These factors included age, body mass index (BMI), education, marital status, smoking, drinking habits, and employment history. The study was undertaken after and with ongoing institutional review board approval. All participants were fully informed about the objective of this study and signed the consent form. Human subject information for this study remains confidential and within the institution.

2.2. 1-Hydroxybenzene (I-OHP) measurement

Urine samples were collected in sterilized 50 ml polypropylene cups right before sperm sample collection. Immediately after collection, samples were stored at −80 °C until analysis. Urinary 1-OHP was analyzed using a high performance liquid chromatography (HPLC) with a fluorescent detector. A 10-ml urine specimen was adjusted first to pH 5.0 with 1.0 N acetic acid. The sample was incubated for 24 h with 15 μl of ß-glucuronidase/sulfatase at 37 °C. A sample purification and enrichment cartridge, packed with C18 reverse-phase liquid chromatograph material, was used to extract the PAH metabolites from urine. Twenty μl of extract was injected into a column of the HPLC system with an auto-injector and a fluorescence detector. Normalized concentrations of urinary 1-OHP