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DNA repair decline during mouse spermiogenesis results in the accumulation of heritable DNA damage

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ABSTRACT

The postmeiotic phase of mouse spermatogenesis (spermiogenesis) is very sensitive to the genomic effects of environmental mutagens because as male germ cells form mature sperm they progressively lose the ability to repair DNA damage. We hypothesized that repeated exposures to mutagens during this repair-deficient phase result in the accumulation of heritable genomic damage in mouse sperm that leads to chromosomal aberrations in zygotes after fertilization. We used a combination of single or fractionated exposures to diepoxybutane (DEB), a component of tobacco smoke, to investigate how differential DNA repair efficiencies during the 3 weeks of spermiogenesis affected the accumulation of DEB-induced heritable damage in early spermatids (21–15 days before fertilization (dbf)), late spermatids (14–8 dbf) and sperm (7–1 dbf). Analysis of chromosomal aberrations in zygotic metaphases using PAINT/DAPI showed that late spermatids and sperm are unable to repair DEB-induced DNA damage as demonstrated by significant increases ($P < 0.001$) in the frequencies of zygotes with chromosomal aberrations. Comparisons between single and fractionated exposures suggested that the DNA repair-deficient window during late spermiogenesis may be less than 2 weeks in the mouse and that during this repair-deficient window there is accumulation of DNA damage in sperm. Finally, the dose–response study in sperm indicated a linear response for both single and repeated exposures. These findings show that the differential DNA repair capacity of postmeiotic male germ cells has a major impact on the risk of paternally transmitted heritable damage and suggest that chronic exposures that may occur in the weeks prior to fertilization because of occupational or lifestyle factors (i.e., smoking) can lead to an accumulation of genetic damage in sperm and result in heritable chromosomal aberrations of paternal origin.

1. Introduction

Spermatogenesis is a complex differentiating system that is initiated from stem cells through division of spermatogonia to form spermatocytes (meiotic cells), which after two meiotic divisions give rise to haploid spermatids (postmeiotic cells) [1]. During the last phase of spermatogenesis, also known as spermiogenesis, haploid spermatids undergo major morphological changes to form mature spermatozoa [2,3]. The somatic and meiotic histones of spermatids are replaced ~14 days before ejaculation in the mouse (~21 days in humans) with basic transition proteins [4] and then with protamines, which

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are arginine-rich proteins that condense the chromatin to a level that is six times more compacted than that observed in mitotic chromosomes [5] and causes the DNA to become transcriptionally inactive and inaccessible to DNA repair proteins [6]. The process requires a profound change in DNA topology that involves the introduction of many single and double strand breaks (DSBs) to eliminate negative supercoiling [2,7]. Transition proteins are thought to play an important role in assuring the proper repair of these breaks by bringing broken DNA fragments into close proximity [5] and there is evidence that they can stimulate DNA repair following genotoxic insults and contribute to maintaining the integrity of the male genome [5,8–10].

It is well established that postmeiotic male germ cells are extremely sensitive to the induction of heritable genomic damage. Over 50 years of germ cell mutagenesis have shown that the majority of mutagens induce their highest effect during the last few weeks of spermatogenesis before fertilization [11,12]. The high sensitivity of the postmeiotic period to mutagenic exposure has been associated with the reduced DNA repair capacity of late spermatids and sperm as compared to early spermatids and other spermatogenic cell types [13–17]. All major DNA repair pathways seem to be less functional in late spermatids and sperm [17–20]. This inability of sperm to repair DNA lesions as they occur may make them particularly susceptible to repeated exposures that take place because of occupational or life style (e.g., tobacco smoking) reasons. These observations suggest that genomic damage induced in late spermatids and sperm may accumulate in the fertilizing sperm and be transmitted to the embryo.

The analysis of paternal chromosomes at the metaphase plate of the first-cleavage division (1-Cl) provides the earliest time after fertilization where paternal chromosomes can be investigated by classic cytogenetic methods [12,21]. At least 16 male germ cell mutagens have been shown to increase chromosomal aberrations in mouse zygotes after exposure of postmeiotic male germ cells [12]. These mutagens have a variety of mechanisms of actions, including DNA alkylation, protein alkylation, and DNA cross-linking, yet, they all produced almost exclusively chromosome-type aberrations, i.e., affecting both sister chromatids, at 1-Cl metaphase. Although it is still unclear why this is the case, these consistent observations of chromosome-type aberrations prove that DSBs are an obligatory step in the processing of sperm lesions into chromosomal aberrations at 1-Cl metaphase. It was postulated that protamine adducts may be the primary type of damage induced in postmeiotic cells by alkylating agents and that adducted protamines create physical stresses in the chromatin structure leading to DSBs before fertilization [22]. Alternatively, adducted protamines may be refractory to removal from DNA during pronuclear formation in the fertilized egg, and thus may indirectly function as “bulky DNA adducts”. Other lesions, such as single strand breaks, base damages and apurinic or apyramidinic sites could be converted into DSBs by misrepair before zygotic S-phase leading chromosome-type aberrations at 1-Cl metaphase [23]. Studies with DNA repair inhibitors in mouse zygotes after sperm treatment with X-rays and chemical agents have provided compelling evidence that chromosomal aberrations were formed after fertilization rather than before [23–25]. Finally, we recently obtained strong evidence that DSBs persisted unrepaired in the sperm for at least 7 days before fertilization (dbf) and that improper repair of transmitted DSBs by the egg leads to increases frequencies of zygotes with aberrations in paternal chromosomes at 1-Cl metaphase [26].

Substantial evidence indicates that tobacco smoking by either parent is associated with increased risk of abnormal pregnancy outcomes. However, little is known of the mechanisms by which tobacco smoking may damage germ cells and affect the developing embryo. Tobacco smoke contains numerous carcinogenic, mutagenic, and reproductive toxicants [27–30]. 1,3-Butadiene (BD) is one of the few constituents of tobacco smoke tested for heritable and developmental effects in laboratory animals [31–34]. BD is classified as a probable human carcinogen [35] and is present in mainstream tobacco smoke at a concentration of 16–75 μg/cigarette and at higher concentrations (205–361 μg/cigarette) in sidestream smoke [36], the main constituent of environmental tobacco smoke or second-hand smoke. BD has marked species differences in susceptibility to the carcinogenic effect, possibly due to differences in metabolism [37–40]. BD is metabolized by the cytochrome P-450-dependent monoxygenases to 1,2-epoxybutene-3 (EB), which is further metabolized by oxidation to diepoxybutane (DEB) [39–41]. DEB is a bifunctional alkylating agent that induces interstrand and intrastrand DNA–DNA cross-links by alkylating two adjacent bases within the major grove of a DNA duplex [42,43] and DNA–protein cross-links [44–46]. DEB is both a somatic and germ cell mutagen in mammals [47–49]. Exposure of male rodents to DEB induces cytogenetic damage in meiotic cells [50] and in zygotes [50,51], as well as dominant lethality and heritable translocations in the offspring [51].

The specific goals of this study were to determine: (i) whether the ability of repairing DEB-induced DNA damage declines as male germ cells progress through spermiogenesis; (ii) whether DEB-induced sperm lesions accumulate during spermiogenesis; (iii) the effective duration of the DNA repair-deficient phase of spermiogenesis after DEB exposure; and, (iv) the dose–response curve for the induction of chromosomal aberrations in zygotes after either single or fractionated DEB exposure of male germ cells.

2. Materials and methods

2.1. *Animals and treatments*

The use of vertebrate animals in these experiments was approved by both the Lawrence Livermore National Laboratory and Lawrence Berkeley National Laboratory Institutional Animal Care and Use Committees. B6C3F1 mice (Harlan Sprague–Dawley Inc., Indianapolis, IN, USA) between 8 and 12 weeks of age were maintained under a 12 h light/12 h dark photoperiod (light from 7:00 a.m. to 7:00 p.m.) at room temperature of 21–23 °C and relative humidity of 50 ± 5%. Food and water were provided *ad libitum*. Male mice were randomly assigned to treat and control experimental groups.

All experimental treatment regimens are shown in Fig. 1. The highest acute DEB dose utilized in this study was selected based on published data [51] and initial experiments in which