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Paternal smoking and germ cell death: A mechanistic link to the effects of cigarette smoke on spermatogenesis and possible long-term sequelae in offspring

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ABSTRACT

Paternal exposure to constituents of cigarette smoke (CS) is reportedly associated with infertility, birth defects and childhood cancers even though the mechanism behind this relationship is still unclear. Chronic cigarette smoking by men leads to poor sperm quality and quantity mainly through oxidative stress and also direct assault by CS metabolites. Among several carcinogenic and teratogenic components of cigarette smoke condensate (CSC), polycyclic aromatic hydrocarbons (PAHs) display a preeminent role in accelerating germ cell death via the cytoplasmic transcription factor, aryl hydrocarbon receptor (AHR) that is present across all stages of spermatogenesis. Activation of AHR by growth factors though benefits normal cellular functions, its mediation by CSC in a spermatocyte cell line [Gc2(spd)ts] adversely affects the expression of a battery of genes associated with antioxidant mechanisms, cell proliferation and apoptosis, and cell cycle progress. Besides, the CSC-mediated cross talk either between AHR and NRF2 or AHR-NRF2 and MAPKs pathways inhibits normal proliferation of the spermatogenic GC-2spd(ts) cells *in vitro* and cell death of spermatocytes *in vivo*. Pharmacological inactivation of CSC-induced AHR but not its genetic manipulation seems preventing DNA and cell membrane damage in Gc2(spd)ts. Data from recent reports suggest that the cigarette smoke affects both the genomic and epigenomic components of the sperm and attributes any associated changes to developmental defects in the offspring. Thus, the studies discussed here in this review shed light on possible mechanistic factors that could probably be responsible for the paternally mediated birth defects in the offspring following exposure to the toxic constituents of cigarette smoke.

1. Introduction

Tobacco use is the single largest preventable cause of death and disease for both men and women. Tobacco causes nearly six million deaths per year worldwide. In the US, smoking and second-hand smoke cause one in every five deaths and incur almost $300 billion annually in total economic costs (USDHHS, 2014). Approximately 30% of women and 35% men of reproductive age smoke cigarettes, affecting not just themselves but also the environment and their progeny (ASRM, 2012). Cigarette smoke (CS) contains more than 7000 chemicals, including at least 539 polycyclic aromatic hydrocarbons (PAHs), of which 69 are proven carcinogens (IARC, 2004; Rodgman and Perfetti, 2009) and mutagens (DeMarini, 2004). Additionally, CS is comprised of the entire top ten hazardous substances listed in section 204 of the Comprehensive Environmental Response, Compensation, and Liability Act. Cigarette smoke condensate (CSC), the particulate, or tar, phase of CS, consists mainly of dioxins (TCDD) and halogenated and nonhalogenated PAHs including benzo(a)pyrene (B[a]P) and pro-oxidants such as lipophilic semiquinones (Smith and Hansch, 2000; Ding et al., 2007). This review on paternal smoking and its impacts on offspring will summarize the current state of research in this area and describe possible mechanisms by which paternal smoking causes poor reproductive outcomes and developmental defects. Moreover, the molecular mechanisms we tried to understand by using a spermatogenic cell line [GC-2spd(ts)] may or may not

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reflect upon the actual events happening in the spermatocytes *in vivo* (See Fig. 1).

1.1. Paternal smoking and developmental defects

Maternal smoking and *in utero* exposure during pregnancy has so far been believed to be associated with reduced sperm quality, count, and testis size in adults (Jensen et al., 2004; Virtanen et al., 2012). However, several epidemiological and case control studies in humans have reported that children born to male smokers are at increased risk of childhood cancers (Ji et al., 1997; Chang et al., 2006; Vine, 1996) and the existence of a significant correlation between paternal smoking and childhood cancer (Liu et al., 2011) with the emphasis on the need to focus on underlying toxicological mechanisms, such as genotoxic, transcriptomic, or epigenomic effects on sperm or cord blood. Similarly, several other reports have highlighted close connection between paternal smoking and childhood leukemia (Pang et al., 2003; Lee et al., 2009). Birth defects such as anorectal malformations (Zwink et al., 2011), cardiovascular anomalies, congenital heart disease (Cresci et al., 2011), cleft palate, hydrocephalus, urethral stenosis (Savitz et al., 1991), spina bifida (Zhang et al., 1992), and reduced kidney volume (Kooijman et al., 2015) were some of the developmental defects observed in the offspring of paternal exposure. Additionally, paternal smoking has been reported to cause implantation failure (Janny and Menezo, 1994; Sofikitis et al., 1995; Ubaldi et al., 1999). However, only a few studies have correlated these outcomes to the intratesticular levels of harmful and potentially harmful constituents of cigarette smoke. Godschalk et al. (2015) recently showed that B[a]P is able to induce hypomethylation in testicular DNA, that leads to heritable mutations in the offspring. However, the data are not very conclusive on the effects of male smoking on *in vitro* fertilization (IVF) outcomes (Pattinson et al., 1991; Hughes et al., 1994; Joesbury et al., 1998). even though the sperm numbers were found decreased in young men prenatally exposed to paternal smoking (Axelsson et al., 2013). So far in humans, the studies on the association between paternal smoking and congenital anomalies among offspring have yielded mixed results. They are either poorly understood mechanistically, and or very limited data are available on the germ cell and reproductive effects of paternal exposure even though maternal exposure to second-hand smoke during pregnancy is known to cause adverse fetal outcomes (Olshan and Faustman, 1993; Leonardi-Bee, and Britton, 2011). In case of rodents, CS-induced mutations in sperm DNA (Yauk et al., 2007) cause lack of pregnancy after fertilization, disrupted blastocyst implantation, impaired embryonic development, and IVF failures (Kapawa et al., 2004).

1.2. Cigarette smoking and male infertility

Even though several studies have indicated the harmful effects of *in utero* exposure to CS on male fertility (Jensen et al., 2004; MacKenzie and Angevine, 1981), chronic cigarette smoking by men also leads to male infertility; the available biologic, experimental, and epidemiological data indicate that 13% of male infertility is attributed to cigarette smoking (ASRM, 2012), and the time to pregnancy is extended in cases in which the man smokes more than 15 cigarettes per day (Ford et al., 2000). A comprehensive review by Ramlau-Hansen et al. (2007) and Mostafa (2010) have provided a thorough overview on cigarette smoking by men and associated abnormalities in sperm count, motility, and morphology, as well as other qualitative and quantitative measures of sperm characteristics. Male smokers exhibit several seminal anomalies including increased levels of oxidative DNA damage (Fraga et al., 1996; Shen et al., 1997), sperm DNA strand breaks (Potts et al., 1999), DNA adducts (Horak et al., 2003), chromosomal abnormalities (Robbins et al., 1997; Rubes et al., 1998), and decreased viability, and fertility (Kunzle et al., 2003). Exposure to cigarette smoke (CS) results in decreased sperm membrane permeability and activity of acrosin (Sofikitis et al., 2000). The testicular endocrine and spermatogenic functions, and epididymal functions are also reportedly reduced in rats upon exposure to B[a]P (Ramesh et al., 2008). Nicotine causes testicular toxicity by degenerating germ cells (Jana et al., 2010) and cigarette smoke metabolites such as cotinine drastically affect seminal parameters such as sperm membrane damage, reduced motility, capacitation and hyperactivation (Pacifici et al., 1993, 1995) that correlates to low sperm count (Chia et al., 1994; Vine et al., 1996). Vine et al. (1994, 1996) reported that sperm concentration is 13% lower in smokers than non-smokers. Meanwhile, there is a modest reduction (10–17%) in sperm counts reported in adult men who smoke heavily and these reductions in sperm quality and quantity are directly proportional to the number of cigarettes smoked daily (Ramlau-Hansen et al., 2007). Such adverse effects of male smoking are thought to be due to absorption of constituents of CS and its metabolites into the systemic circulation and accumulation, either by diffusion or active transport, into seminal plasma (Zavos and Zarmakoupis-Zavos, 1999).

Several environmental and food contaminants are known to reach the testis in significant concentrations (Gaspari et al., 2003; Bjorge et al., 1996). However, little is known about the molecular mechanisms by which the components of CS damage male germ cells during spermatogenesis. One likely candidate is the oxidative stress caused by the generation of excess reactive oxygen species (ROS) or free oxygen radicals by the toxic constituents of CS (Saleh et al., 2002; Aitken and Baker, 2004). Additionally, the sperm of smokers have increased levels of oxidized unsaturated fatty acids (Jones et al., 1979). Although ROS are required for sperm maturation, capacitation, and the acrosome reaction (de Lamirande and Gagnon, 1993), mature male gametes are highly susceptible to oxidative damage because they express low levels of antioxidant enzymes and have high concentrations of polyunsaturated fatty acids in their plasma membrane (Aitken and Roman, 2008). Cigarette smokers with high levels of ROS in their seminal plasma capable of causing DNA damage mediate oxidative male infertility (Potts et al., 1999; Tremellen, 2008). Meanwhile, infertile men who smoke cigarettes have higher levels of seminal OS than infertile nonsmokers and significantly low sperm count (Saleh et al., 2002; Collodel et al., 2010; Zenses, 2000). Therefore, the oxidative imbalance could be, in part, responsible for CS-mediated male infertility. Genetically, smoking has been known to be associated with sperm disomy in teenage men (Rubes et al., 1998). Smoking also affects morphology and ultrastructure of the flagellum and, more specifically, the axoneme of the human spermatozoon (Evans et al., 1981; Hoidas et al., 1985; Zavos et al., 1998). In addition, the change mediated by CS in sperm mRNA profile can serve as the marker of gene–environmental toxicants interactions in human germ cells (Linschooten et al., 2009). In contrast, studies by Mocarelli et al. (2011) showed that only *in utero* and lactational exposure of children to low doses of TCDD could permanently reduce sperm quality. Meanwhile, a meta-analysis by Li et al. (2011) have highlighted that the smoking seems to degrade semen volume and total sperm count in heavy smokers.

Polycyclic aromatic hydrocarbons (PAHs) act as testicular toxicants through aryl hydrocarbon receptor (AHR).

Treating adult rodents with PAHs such as TCDD, B[a]P, and 3-Methylchloranthrene increases the number of abnormal sperm and immature germ cells (Viczian, 1968; Wyrobeck and Bruce, 1975), blocks spermatogenesis, and causes testicular atrophy (Mattison, 1982), decreased testis weight, and increased apoptosis