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Full Length Article

Urban fine particulate matter exposure causes male reproductive injury through destroying blood-testis barrier (BTB) integrity

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HIGHLIGHTS

- $PM_{2.5}$ was exposed to male rats at dose levels of 0, 10, and 20 mg/kg.b.w-day.
- Major spermatogenesis marker protein expressions reduced after treatment.
- SOD activity and expression levels enhanced after treatment.
- Sertoli cell oxidative stress and apoptosis were observed after treatment.
- Major BTB junction protein expressions reduced after treatment.

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ABSTRACT

Blood-testis barrier (BTB) provides a suitable microenvironment for germ cells that is required for spermatogenesis. Exposure to particulate matter (PM) is recognized to occasion male reproductive impairment, but the mechanism of which remains unclear. Male Sprague-Dawley (SD) rats were used to establish animal models with $PM_{2.5}$ exposure concentration of 0, 10, and 20 mg/kg.b.w. once a day for four weeks. Success rate of mating, sperm quality, epididymal morphology, expressions of spermatogenesis markers, superoxide dismutases (SOD) activity and expression in testicular tissues, and expressions of BTB junction proteins were detected. In addition, in vitro experiments were also performed. After $PM_{2.5}$ treatment, reactive oxygen species (ROS) production and apoptosis of Sertoli cells were analyzed. Our results indicated that after $PM_{2.5}$ exposure male rats presented inferior uberty and sperm quality, with decreased expressions of spermatogenesis markers, escalated SOD activity and expression levels, and reduced expressions of tight junction, adherens junction, and gap junction proteins in testicular tissues. Meantime, $PM_{2.5}$-treated Sertoli cells displayed increased SOD production and apoptosis. $PM_{2.5}$ exposure engenders male reproductive function injury through breaking BTB integrity.

1. Introduction

Maintenance of normal male fertility relies on sufficient sperm production, thus stability of spermatogenesis microenvironment played a pivotal role. The blood-testis barrier (BTB) is constituted mainly by tight junctions, adherens junctions, and gap junctions between adjoining Sertoli cells near the basement membrane of seminiferous epithelium in mammalian testis (Gerber et al. 2016). Each junction type performs irreplaceable physiological function. Tight junctions confer cell polarity and form an impervious barrier

Abbreviations: PM2.5, fine particulate matter; ROS, reactive oxygen species; NS, normal saline; Cx43, connexin 43; SOD, superoxide dismutase; BTB, Blood-testis barrier; SD, Sprague-Dawley; OD, optical density; HE, hematoxylin and eosin; FCS, fetal calf serum; DCFH-DA, 2′,7′-dichlorofluorescin-diacetate; PBS, phosphate-buffered saline; ZO-1, zonula occludens-1; β-catenin, beta catenin; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride; DMC1, dosage suppressor of mck1; Plzf, promyelocytic leukemia zinc finger; SCP3, synaptonemal complex protein 3; Stra8, stimulated by retinoic acid gene 8; GLM, general linear model.

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that restrains seepage of water, ions and other molecules between cells (Cereijido et al., 1998). Adherens junctions create an uninterrupted adhesions vinculum beneath tight junctions to connect adjacent cells together, except where adherens junctions and tight junctions coexist (Mruk and Cheng, 2004). While gap junctions regulate spermatogenesis and gear up intercellular communication (Weider et al., 2011).

BTB isolates spermatocytes and spermatids away from circulatory and lymph system, building a condign biochemical and immunological microenvironment that is indispensable for their development (Dym and Fawcett, 1970; Setchell, 2008), curtaining meiotic and haploid germ cells from being recognized and attacked by the host immune system due to a number of specific antigens are provisionally expressed in them, in addition, blocking detrimental substances from entering into the microenvironment (Mruk et al., 2008; Cheng and Mruk 2010). BTB plays a critical role in the onset and upkeep of spermatogenesis. Occludins are acknowledged to oligomerize to form tight junction strands through homologous and heterologous mutual effect of occludins localized on neighboring Sertoli cells (Blasig et al., 2011). In occludin(−/−)mice, tight junction fibrils failed to congregate, and the mice with malfunctional BTB presented sterile (Saitou et al., 2000). And spermatogenesis of claudin-11(−/−) mice was not able to advance beyond meiosis (Gow et al., 1999). Connexin43 (Cx43) functions crucially in germ cell development for loss of Cx43 issues in a reduced germ cell number in fetus and a thorough spermatogenesis suppression in the adult Sertoli cells (Sridharan et al., 2007; Kidder and Cyr, 2016). It had been indicated that there was association between elevated cell adhesions and gap junction proteins expression and Spermatogonial stem cells (SSCs) differentiation induced by BMP4 (bone morphogenetic protein 4) (Carlomagno et al., 2010), supporting the hypothesis that an integral BTB furnishes and sustains an appropriate microenvironment to ensure spermatogonial differentiation.

As the key component of air pollution that occurs anywhere, ambient fine particulate matter ($PM_{2.5}$) has become a widespread public health concern. Convincing evidences have demonstrated a positive correlation between $PM_{2.5}$ and increased disease-related events in humans (Beelen et al., 2008; Sack and Goss 2015). Infaust impact of particulate matter (PM) on male fertility has been growingly emphasized in recent years, and PM exposure has been considered to bring about reduced sperm quality of males (Hammoud et al., 2010; Zhou et al., 2014). An investigation executed on rats reflected that PM affects the process of spermatogenesis and sperm parameters (Ahmed et al., 2013). Study of Omurtag et al. showed that PM exposure altered energy homeostasis in early stages of male germ cell development and aroused decrease of sperm motility (Omurtag et al., 2015). Polyzos et al. held the attitude that mice exposed to PM may be affected on sperm motility and fertilization rates (Polyzos et al., 2009). Rubes et al. reported that PM-derived air pollution may result in sperm DNA damage and raised the rates of male-mediated infertility (Rubes et al., 2005). Although the unfavorable influence of PM on male fertility has been well documented, specific mechanisms are still not illustrated adequately.

Oxidative stress has acted as a fundamental mechanism of PM-connected impairment in accord with plenty of publications, which appears on the condition that reactive oxygen species (ROS) generation surpasses the scavenging ability of antioxidant defense system (Sun et al., 2002). Nucleobase levels increased in human circulating blood cells and urine could be traced back to oxidative injury after PM exposure (Moller et al., 2014). Intervention studies on lung epithelial cells using antioxidants revealed that cigarette smoke-mediated oxidative stress raised ROS levels and caused DNA damage (Faux et al., 2009). Data from Cui et al. noted that amount of circulating endothelial progenitor cells lessened in mice after PM treatment on account of redox-related ROS formation (Cui et al., 2015). For male infertility, oxidative mechanisms are of special relevance in that high concentrations of polyunsaturated fatty acids in sperm plasma membrane are immensely vulnerable to oxidative lesion, resulting in integrity collapse of the sperm membrane (Aitken et al., 1989). Farther, sperm nucleus is absent of resistibility to oxidative stress and is susceptible to DNA scathe elicited by oxidation (Simon and Carrell, 2013). Hence, oxidative stress probably acts as an important role in PM-induced semen damage of males.

Since an intact and functional BTB provides a suitable microenvironment and guarantees the normal proceeding of spermatogenesis, we have reasons to doubt whether possible male reproductive impairment caused by $PM_{2.5}$ breaks BTB integrity and the microenvironment, and intervenes sperm generation. Meanwhile, we speculate that oxidative stress is involved in the aberrant spermatogenesis.

Exposure to ambient particulate matter associated with air pollution is an inevitable lifelong jeopardy for all of us. It is thereby eventful to pinpoint the principium of $PM_{2.5}$ pathopoiesis. The present research focuses upon the potential mechanism of $PM_{2.5}$-originated male infertility concerning alteration of BTB integrity.

2. Methods

2.1. Ethics statement

All experiments involving animals were approved by the Ethics Committee of Chongqing Medical University (license numbers: SCXK[YU]20110016).

2.2. $PM_{2.5}$ samples

Sampling method we use was in accordance with our precious research (Cao et al., 2015). A Thermo Anderson G-2.5 air sampler (Model GV 2630 Series, USA) was used to collect particulate matters from March 2015 to September 2015 in main urban street of Chongqing, China, PM where has been reported to arouse declined sperm quality in males (Zhou et al., 2014). $PM_{2.5}$ collected contains multiple metals of which the most ample are aluminum, iron, zinc, copper, lead, titanium and manganese according to elemental analysis from Chongqing Environmental Monitoring Center.

2.3. Animals and treatments

Thirty-nine male Sprague-Dawley (SD) rats (six weeks old, 113–179 g) were provided by Experimental Animal Center of Chongqing Medical University. The protocol was approved by the Animal Experimentation Ethical Committee of Chongqing Medical University. In this research, rats had free access to food and water, and were kept under the specific pathogen-free condition (12 h light/12 h dark cycle with humidity of $55 \pm 5\%$ at $25 \pm 2\,^{\circ}C$). Rats were divided into three groups randomly (n = 13).

Filters were sheared into pieces and sonicated with an ultrasonator (KQ-250DE, Shumei, China) for 3×40 min in 0.9% saline after sampling. A Thermo Scientific Power Dry LL3000 vacuum-freeze dryer (USA) was used to dry PM suspension and the farinose solid got was weighed and stored at $-20\,^{\circ}C$. Before use, farinose solid was diluted with 0.9% sterilized saline to the concentration needed. $PM_{2.5}$ exposure doses in our previous research were 9 mg/kg.b.w. and 24 mg/kg.b.w. (0.15 mL/100 g.b.w.), which proved to be successful in animal modeling (Cao et al., 2015). We chose $PM_{2.5}$ exposure dose of 10 mg/kg.b.w. and 20 mg/kg.b.w. (0.15 mL/100 g.b.w.) to perform in this study, which