Influence of PM$_{2.5}$ on spermatogenesis dysfunction via the reactive-oxygen-species-mediated Mitogen-activated-protein-kinase signaling pathway

**Abstract**

Approximately 15% of the world’s couples confront childlessness, and about 50% of them are due to male reproductive disorders. Several previous studies demonstrated that PM$_{2.5}$ particles have been consistently associated with critical human sperm reduction and impairment of human sperm chromatin and DNA from traffic exhaust pollution. Blood-testis barrier (BTB), a critically physical barrier between the seminiferous tubules and the blood vessels prevents sperm antigens from entering the blood circulation and facilitating and initiating an autoimmune response that contributing to spermatogenesis interference. Reactive oxygen species (ROS) are involved in the redox-sensitive signal transduction factors activation, such as Jun NH$_{2}$-terminal kinase (JNK), p 38, extracellular signal-regulated kinase (ERK), and mitogen-activated protein kinases (MAPK) that critically influence BTB disruption. After PM$_{2.5}$ exposure, there are decreased superoxide dismutase (SOD) expression, increased malondialdehyde (MDA) expression, increased nuclear factor erythroid 2-related factor 2 (Nrf2) expression, increased expression of the four junctional proteins (β-catenin, Cx43, occludin, zona occludens-1 (ZO-1)), thus improve sperm quality and quantity, PM$_{2.5}$ particles markedly induce increasing phosphorylation of MAPKs via the ROS-mediated MAPK signaling pathway that causes BTB disruption, but this effect is lesser in the vitamins C and E intervention as well as increasing oxidative stress and cell apoptosis, and prevent toxic effects.

**Keywords:** PM$_{2.5}$, spermatogenesis, ROS, MAPK

**Abbreviations:** BTB, Blood-Testis Barrier; Cx43, Connexin 43; DNA, Deoxyribonucleic Acid; ESs, Ectoplasmic Specializations; ERK, Extracellular signal-Regulated Kinase; GJs, Gap Junctions; JNK, Jun NH$_{2}$-terminal Kinase; Nrf2, Nuclear factor erythroid 2-related factor 2; MAPK, Mitogen-Activated Protein Kinase; MDA, Malondialdehyde; PM$_{2.5}$, Particulate Matter with diameter of 2.5 microns or less; ROS, Reactive Oxygen Species, SOD, Superoxide Dismutase; TJs, Tight Junctions; ZO-1, Zonula Occludens-1

**Introduction**

Globally, ambient fine particulate matter (PM$_{2.5}$) exposure has been an intractable health problem and associated with increased morbidity or mortality due to widespread excessive discharge of automobile exhaust and industrial development.1-6 PM$_{2.5}$ can carry harmful and toxic substances deeply into the lungs and blood circulation, contributing damage to multiple organs, including reproductive system,7 endocrine system,8 nervous system.9 Epidemiological data have demonstrated that approximately 15% of the world’s couples confront childlessness, and around 50% of these cases are due to male reproductive problems.10,11 Previous studies revealed that PM$_{2.5}$ has consistently been related to significant sperm quality reduction,12,13 as well as traffic exhaust pollution impairment of human sperm chromatin and deoxyribonucleic acid (DNA).14

**Blood-Testis Barrier**

The integrity of the blood-testis barrier (BTB) is critical for spermatogenesis in addition to the production of a large amount of sperm for maintenance of male reproductive function.15,16 BTB, a physical barrier between the seminiferous tubules in the testes and the blood vessels, not only isolates harmful substances accessing to the seminiferous tubules, but also prevents sperm antigens from entering the blood stream and initiating an autoimmune response that interfering with spermatogenesis.15,16 In mammals, BTB is one of the tightest blood-tissue barrier. BTB composes desmosomes between Sertoli cells, tight junctions (TJs), gap junctions (GJs), and basal ectoplasmic specializations (ESs).17 ESs, characteristic adhesion proteins main components of N-cadherin and β-catenin attached to testis, are the most typical and prominent ultrastructural features of the BTB.18 GJs with an import GJ component “connexin 43 (Cx43) compose of gap junction proteins through docking with semi-channels between Sertoli cells and between Sertoli cells and germ cells,”19 whereas TJs with important components “occluding and zona odculdens-1 (ZO-1)” are formed by connective tissue structures between adjacent Sertoli cells with selective permeability.20 Some previous studies demonstrated that after atmospheric PM$_{2.5}$ exposure, the BTB was severely damage, resulting in spermatogenesis dysfunction and infertility.20,21 Nevertheless, very few studies have investigated on the mechanism underlying these hazardous effects.22,23

**Reactive oxygen species and mitogen-activated protein kinases**

Reactive oxygen species (ROS) in the body are normally in a dynamic equilibrium association with antioxidants. PM$_{2.5}$ exposure induces excessive ROS production that is hazardous to male...
reproductive system. Several previous studies demonstrated that ROS are involved in the redox-sensitive signal transduction factors activation, such as Jun NH2-terminal kinase (JNK), p38 extracellular signal-regulated kinase (ERK), and mitogen-activated protein kinases (MAPKs). BBT disruption is critically depended on these kinases. MAPK pathway can activate Nrf-2, a transcription factor and the main regulator of antioxidant response elements that regulates antioxidant induction. Spermatogenesis dysfunction has been caused by oxidative stress, whereas vitamins C and E have been demonstrated to reduce the impacts of environmentally toxic substances on the testes. Furthermore, very few studies have investigated the beneficial effects of vitamins C and E on PM2.5-exposed spermatogenesis dysfunction, particularly from vehicle-exhaust-originated PM2.5 exposures.

**Discussion**

Differences in the composition of PM2.5 particles collected at different times and in different areas may contribute to diverse study results in human spermatogenesis although atmospheric PM2.5 particles have been confirmed to significantly impair male spermatogenesis. Critically, the BBT is a barrier between the seminiferous tubules and the blood vessels. The BBT plays a significant role in protecting germ cells in the testes from toxic substances in the environment. PM2.5 from automobile exhaust facilitates spermatogenesis dysfunction via disruption of the BBT through the ROS-mediated MAPK signaling pathway. Combined vitamins C and E intervention effectively reduces PM2.5-induced toxicity in the male reproductive system. Oxidative stress is related to significantly decreased effectiveness of antioxidant defenses or increased ROS production. Severe and persistent oxidative stress can trigger cell apoptosis. After PM2.5 exposure, superoxide dismutase (SOD) expression is significantly decreased, whereas malondialdehyde (MDA) and nuclear factor erythroid 2-related factor 2 (Nrf-2) expressions are significantly increased, indicating that excessive oxidative stress in the testes contributes to reduced SOD expression. Vitamins C and E can restore seminiferous tubule shape and cell layers of spermatogonia, spermatocytes, spermatids, and Sertoli cells as well as increases the expression of the four functional proteins (β-catenin, Cx43, occludin, zonula occludens-1 (ZO-1)), thus improve sperm quality and quantity. After PM2.5 exposure, there is markedly increase of the phosphorylation of MAPKs via the ROS-mediated MAPK signaling pathway that causes BBT disruption, but this effect is lesser in the vitamins C and E intervention. Additionally, after PM2.5 exposure, cleaved caspase-3 expression is increased and the Bel-2/Bax ratio is decreased.

**Conclusion**

PM2.5 particles disrupt BTB integrity by reduction of the expression of BTB-associated proteins, such as β-catenin, Cx43, occludin, and ZO-1. The BTB integrity is closely associated with the ROS-mediated MAPK signaling pathway. Combined therapeutic administration of vitamins C and E can maintain the BTB integrity, reduce oxidative stress and cell apoptosis, and prevent toxic effects.

**Funding**

The authors disclose no funding sources.

**Acknowledgments**

Dr. Attapon Cheepsattayakorn conducted the study framework and wrote the manuscript. Associate Professor Dr. Ruangrong Cheepsattayakorn contributed to scientific content and assistance in manuscript writing. Both authors read and approved the final version of the manuscript.

**Conflicts of interest**

The authors declare that they have no actual or potential competing financial interests.

**References**


