— Minireview —

The Effects of Diesel Exhaust on Murine Male Reproductive Function

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Several reports have suggested that semen quality in normal men is declining. Given the lack of consensus about the effects of diesel exhaust (DE) on the male reproductive system, we conducted various experiments. We examined the effect of the exposure of mature male mice to DE for 6 months on the male reproductive system. Daily sperm production per gram from the testes dose–dependently decreased with exposure to DE. Next, we investigated the effect of exposure of pregnant mice to DE on male gonad development at the level of mRNA expression. Expression of mRNAs for steroidogenic factor (Ad4BP/SF-1) and Müllerian inhibiting substance (MIS) decreased significantly in male fetuses when maternal mice were exposed to DE for 8 hr per day between days 2 and 13 post coitum. In addition, DE exposure during the fetal period may have some influence on the male reproductive function in newborn mice. *In utero* DE exposure caused testosterone levels to increase in newborn male mice at age 4 weeks. These findings indicate that exposure to DE may influence the male reproductive system. Further studies are necessary to elucidate the mechanism of the effects of DE on the male reproductive system and to define which classes of compounds are responsible for the changes in the male reproductive system.

Key words ——— diesel exhaust, male reproduction, endocrine disrupting chemical, testis, gene expression, maternal exposure

INTRODUCTION

The number of environmental chemicals known to affect human health by disrupting normal endocrine function through interactions with hormone receptors may continue to increase.[1)] Diesel exhaust (DE) is a complex mixture of particulate and vapor-phase compounds. The soluble organic fraction (SOF) of the particulate materials in diesel exhaust comprises thousands of compounds, including a variety of polycyclic aromatic hydrocarbons (PAHs) and trace amounts of heavy metals.[2,3)] It has been reported that DE and diesel exhaust particles (DEP) are hazardous to human health and may cause cancer,[4)] allergic rhinitis, asthma,[5)] and cardiovascular disease.[6)] It is known that organic compounds adsorbed on these particles are lipophilic and are therefore able to pass through various barriers and membrane systems.

In recent years, there has been growing concern over the effects of exogenous chemicals that exhibit "endocrine disrupting chemicals (EDCs)" or "environmental hormones."[7,8)] This class of chemicals may be producing adverse effects in humans and wildlife by directly or indirectly disrupting the endocrine system through mimicking or antagonizing natural hormones. Possible effects of EDCs include reproductive and developmental abnormalities, increases in certain hormone-related cancers such as breast and prostate, immune system deficiencies, and declines in wildlife populations.[9)] The endocrine disrupting effects of the DEP samples were examined by luciferase reporter assay, *in vitro*.[10)] Therefore, we examined the influence of DE on the murine reproductive system.

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Direct Exposure to DE Affects the Male Mouse Reproductive System[11)]

Heavy metals and pesticides, such as DDT and dibromochloropropane, are suggested to influence the male reproductive system.[12–14)] However, air pollution effects on the male reproductive system have been ignored. Therefore, this study has focused on the influence of DE (one air pollutant substance) on the murine male reproductive system. We determined the concentration of DEP from the WHO-recommended limit of total suspended particles (TSP) in air: 60–90 μg/m^3 (annual mean concentration) or 150–230 μg/m^3 (daily mean concentration).[15)] Since the average DEP content in TSP is approximately 45% in Tokyo, certain areas of Tokyo sometimes exceed 135 μg DEP/m^3.

We have studied the influence of DE on the murine male reproductive system. We used male ICR mice obtained from Japan Clea Co. (Tokyo, Japan). Male mice at 6 weeks of age were exposed to DE for 12 hr (22:00–10:00) a day for 6 months in a 2.3-m^3 chamber. Mice were divided into 4 groups (control and three DE-treated groups) in different chambers and exposed to DE at DEP concentrations of 0.3, 1.0 or 3.0 mg DEP/m^3, or to clean air as a control. We observed that DE has the potential to influence the male reproductive system through effects such as inducing Leydig cell degeneration, increasing the number of damaged seminiferous tubules, and reducing daily sperm production in the testis.

In all testes of mice treated with DE, interstitial edema was observed by light microscopy. Seminiferous tubules in DE-exposed groups showed degenerative and necrotic changes, desquamation of the seminiferous epithelium, and loss of spermatozoa. The damaged tubules were scattered randomly throughout the testis. These effects were dose–dependent and significantly higher in DE-exposed groups than in controls (Fig. 1). DSP per gram testis was significantly decreased in a dose–dependent manner in DE-exposed mice. The DSP/g of DE-exposed mice decreased by 29% in the 0.3 mg DEP/m^3 group ($p < 0.01$), by 36% in the 1.0 mg DEP/m^3 group ($p < 0.01$), and by 53% in the 3.0 mg DEP/m^3 group ($p < 0.01$) (Fig. 2). The recovery from spermatogenesis damage was investigated. After exposure to DE for 6 months, the mice were exposed to clean air for 1 month. Although partial recovery was observed, inhibition of spermatogenesis remained. In the 3.0 mg DEP/m^3 group the DSP/g was still suppressed by 29%.

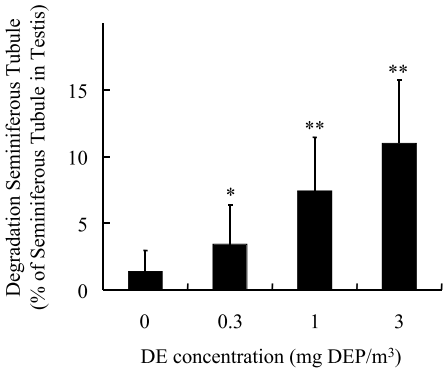

Fig. 1. The Percentage of Degeneration of Seminiferous Tubules in Testicular Cross Sections of Control Mice and Mice Exposed to DE

Estimation of testicular damage was performed by counting the number of tubular cross sections and determining the percentage with a degenerative epithelium in 3 cross sections per testis. Values are means ± S.D. *$p < 0.05$, **$p < 0.01$, compared with controls.

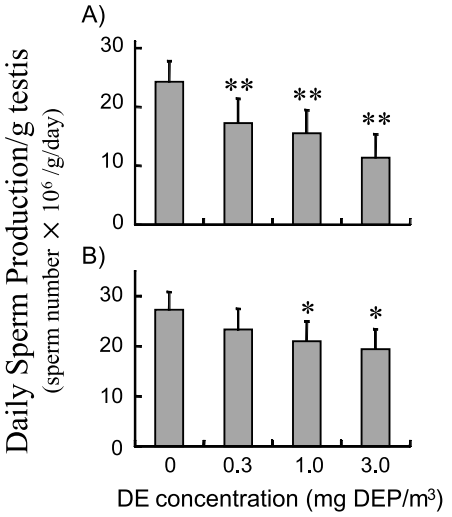

Fig. 2. Daily Sperm Production (DSP) in Mice Exposed to DE for 6 Months (A) and then Exposed to Clean Air for 1 Month (B)

Values are means ± S.D. **$p < 0.01$, compared with controls.

Partial recovery from spermatogenic damage was observed when mice were exposed to fresh air for 1 month following the exposure to DE for 6 months. However, damage to spermatogenesis following DE exposure might not be irreversible.

In this study, we do not know whether DE poses a significant risk to the human male reproductive system. We lack accurate data on the levels of human exposure and whether adverse reproductive changes can be induced by DE in men. However, our data derived from the murine reproductive system suggests that DE may potentially influence sper-

matogenesis in men. Further studies are necessary to define which classes of compounds are responsible for the changes in testicular function.

In utero Exposure to DE Decreases The Expression of Mrnas Essential for Gonad Development in Male Mouse Fetuses[16)]

Normal development of the testes and male genital tract is a tightly regulated process dependent upon a coordinated cascade of molecular and morphological events, including formation of the testes, regression of the Müllerian ducts, and stabilization of the Wolffian ducts. The sex determining gene located on the Y chromosome (SRY) has been shown to encode a testis-determining factor.[17)] In the presence of an active copy of SRY, the undifferentiated gonad forms a testis within which Sertoli cells synthesize and secrete Müllerian inhibiting substance (MIS), which is required for regression of the Müllerian ducts.[18)] MIS acts downstream of steroidogenic factor-1 (Ad4BP/SF-1) and is required for normal male reproductive tract development.[19)] Ad4BP/SF-1, a member of the steroid receptor superfamily of transcription factors, influences reproductive function through the regulation of development and differentiation of hormone producing tissues.[20)] Ad4BP/SF-1 was identified as a steroidogenic tissue-specific transcription factor regulating the expression of the steroidogenic cytochrome P450 genes.[21)]

We investigated the effects of DE exposure of pregnant mice on male gonad development at the mRNA expression level. We used pregnant ICR mice obtained from SLC Co. (Shizuoka, Japan). Exposure began on day 2 post coitum (p.c., the day the plug was found was taken as day 0 p.c.) and continued until day 13 p.c. Animals were exposed for 8 hr per day, and exposed to DE at DEP concentrations of 0.1 or 3.0 mg DEP/m^3, or clean air as a control.

The relative levels of Ad4BP/SF-1 and MIS mRNA were significantly decreased in a dose–dependent manner in DE-exposed male fetuses. Levels of Ad4BP/SF-1 and MIS mRNAs decreased by 30% in the 0.1 mg DEP/m^3 ($p < 0.01$), and by 50% in the 3.0 mg DEP/m^3 ($p < 0.01$) (Fig. 3). Ad4BP/SF-1 has been reported to control the transcription of P450 genes related to steroid synthesis. Therefore, we investigated the influence of DE on the expression of steroidogenic cytochrome P450 genes [P450scc, 3β-hydroxysteroid dehydrogenase (HSD), P450c17, 17β-HSD, and aromatase] in the male fetus. In the 3.0 mg DEP/m^3 group, 3β-HSD and

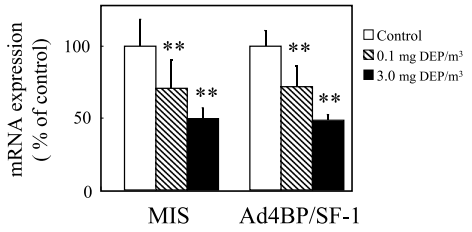

Fig. 3. Effect of Maternal Exposure to DE on MIS and Ad4BP/SF-1 mRNA Levels in Male Fetuses

The ratios were normalized such that the mean ratio of the control is 100%. Values are expressed as means ± S.D. $^{**}p < 0.01$, compared with controls.

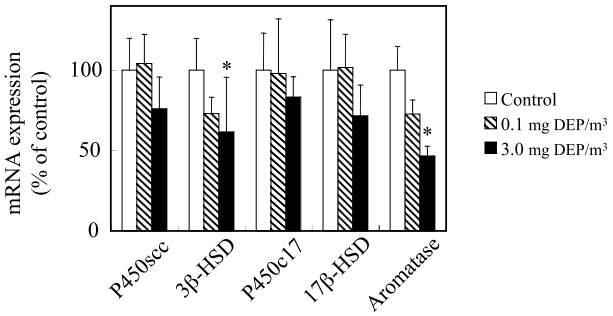

Fig. 4. Effect of Maternal Exposure to DE on Steroidogenic P450 Gene mRNA Levels in Male Fetuses

The ratios were normalized such that the mean ratio of the control is 100%. Values are expressed as means ± S.D. $^{*}p < 0.05$, compared with controls.

aromatase mRNA levels were reduced significantly ($p < 0.01$), and levels of other mRNAs were reduced slightly (Fig. 4).

The main finding of the present study was that exposure of pregnant mice to DE may influence male genital organogenesis by reducing the expression of genes essential to normal gonadal development. However, it is unclear which substances in the DE are responsible for the observed changes. Further studies are required to identify the substances that decrease the expression of these genes and cause the observed developmental defects.

In utero Exposure to DE on the Genital Tract of Male Newborn Mice

To examine the influence of fetal-period DE exposure on natal mice, male embryos were exposed to DE from day 2 p.c. to day 16 p.c. at a DEP concentration of 0.3, 1.0 or 3.0 mg DEP/m^3. After exposure, the authors examined tissue weight, blood hormone levels, and mRNA expression of testis genes in 4-week-old newborn male mice.

Tissue weight of the testis and accessory repro-

Table 1. Effect of Maternal Exposure to DE on Tissue Weight and Blood Testosterone Concentration

	Control	0.3 mg DEP/m^3	1.0 mg DEP/m^3	3.0 mg DEP/m^3
Testis (mg)	42.9 ± 8.9	48.6 ± 16.9	60.8 ± 12.4**	57.8 ± 12.8**
Prostate (mg)	4.3 ± 1.8	6.0 ± 3.2	9.2 ± 6.8*	7.5 ± 3.4*
Coagulating gland (mg)	4.8 ± 2.4	9.2 ± 5.6	10.0 ± 4.2**	9.5 ± 4.4*
Seminal vesicle (mg)	11.3 ± 5.1	20.4 ± 17.6	22.5 ± 10.5	23.8 ± 11.0**
Testosterone (ng/ml)	0.16 ± 0.18	1.37 ± 1.74	1.74 ± 1.68**	1.59 ± 2.10

Values are means ± S.D. *$p < 0.05$, **$p < 0.01$, compared with controls.

ductive glands (prostate, coagulating gland and seminal vesicle) were significantly greater in the DE-exposed groups than in the control group (Table 1). The blood testosterone concentration of mice exposed to 1.0 mg DEP/m^3 was about 8 times higher than that of the control group. However, there were no significant differences in the expression levels of testosterone synthetase mRNA between DE-exposed and control mice. It was suggested that DE exposure during the fetal period has some influence on the steroid synthesis system and male genital system of newborn mice.

CONCLUSION

In this review, the authors focused on the effects of DE on the male reproductive system. The evidence suggests that exposure of mice to DE may influence the male reproductive system. However, this evidence does not suggest whether DE poses a significant risk to the human male reproductive system. We lack accurate data on the levels of human exposure and whether adverse reproductive changes can be induced by DE in men. Recently, De Rosa *et al*. have shown that continuous exposure to traffic pollutants impairs sperm quality in young/middle-aged men.[22] Further studies are required to identify the specific modifications induced by DE on the male reproductive system.

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