

## 细颗粒物对雄性小鼠生殖功能的影响

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**【摘要】目的** 探讨长期环境细颗粒物(PM2.5)暴露对雄性BALB/c小鼠生殖功能的影响。**方法** 40只BALB/c小鼠利用随机数表法随机分为对照组和实验组各20只,对照组和实验组小鼠分别暴露于0.9%的生理盐水雾化、PM2.5混悬液雾化中,两组小鼠均连续暴露12周,每天6 h,随后行睾丸组织病理学检查、精子计数和畸形率测定以及睾丸组织丙二醛(MDA)含量和总抗氧化能力(TAC)检测;此外每组选10只雄鼠与正常雌鼠交配并统计受孕率。**结果** 对照组小鼠睾丸组织结构完整,少量淋巴细胞浸润,实验组小鼠睾丸组织炎性细胞浸润明显,生精小管结构发生明显改变,生精细胞层减少,生精细胞结构排列紊乱;实验组小鼠的精子数量为( $4.43\pm0.55$ ) $\times10^7/mL$ ,与对照组精子数量( $6.47\pm1.02$ ) $\times10^7/mL$ 相比明显下降,差异具有统计学意义( $P<0.05$ );实验组小鼠的精子畸形率为( $3.87\pm0.60$ ),明显高于对照组的( $1.56\pm0.13$ ),差异具有统计学意义( $P<0.05$ );实验组小鼠的睾丸组织中的MDA为( $2.55\pm0.14$ )U/mg,明显高于对照组的( $1.53\pm0.28$ )U/mg,TAC含量为( $13.95\pm1.34$ )U/mg,明显低于对照组的( $18.58\pm2.50$ )U/mg,差异均具有统计学意义( $P<0.05$ );实验组雌鼠受孕率为60.0%,与对照组的100.0%相比明显降低,差异具有统计学意义( $P<0.05$ )。**结论** PM2.5长期暴露能够损伤雄性BALB/c小鼠的生殖功能。

**【关键词】** 细颗粒物2.5;小鼠;生殖功能;生殖毒性;氧化损伤

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**Effect of fine particles on reproductive function of male mice.** ZHOU Feng-hai<sup>1</sup>, ZHOU Chuan<sup>2</sup>, LV Hai-di<sup>1</sup>, LI Hai-yuan<sup>1</sup>. 1. Department of Urology, Gansu Provincial Hospital, Lanzhou 730000, Gansu, CHINA; 2. Department of Urology Surgery, West China School of Medicine, Sichuan University, Chengdu 610000, Sichuan, CHINA

**[Abstract]** **Objective** To explore the effects of long-term exposure to particulate matter 2.5 (PM2.5) on reproductive function in male BALB/c mice. **Methods** A total of 40 BALB/c mice were randomly divided into control group and experimental group according to the random number table method, with 20 mice in each group. Mice in the control group and the experimental group were exposed to atomized 0.9% normal saline solution and PM2.5 suspension, respectively. Both groups of mice were exposed continuously for 12 weeks, 6 hours a day. Subsequent tests were performed or counted, including testicular histopathology, sperm count and malformation rate, the content of testicular tissue malondialdehyde (MDA), and total antioxidant capacity (TAC). In addition, 10 male BALB/c mice in each group were mated with normal female BALB/c mice, and the pregnancy rate was counted. **Results** In the control group, the testicular tissue structure was intact with a small amount of lymphocytes infiltration. In the experimental group, the inflammatory cells infiltration was obviously showed in testicular tissue, the spermatogenic tubule structure changed significantly, the spermatogenic cell layer decreased, and the spermatogenic cell structure was disordered. The sperm number of mice in the experimental group was ( $4.43\pm0.55$ ) $\times10^7/mL$ , which was significantly lower than ( $6.47\pm1.02$ ) $\times10^7/mL$  of mice in the control group, and the difference was statistically significant ( $P<0.05$ ). The sperm malformation rate of the experimental group was ( $3.87\pm0.60$ ), which was significantly higher than ( $1.56\pm0.13$ )% of the control group, and the difference was statistically significant ( $P<0.05$ ). In the testicular tissue of the experimental group, the content of MDA was ( $2.55\pm0.14$ ) U/mg, which was significantly higher than ( $1.53\pm0.28$ ) U/mg of the control group; and the TAC content was ( $13.95\pm1.34$ ) U/mg, which was significantly lower than ( $18.58\pm2.50$ ) U/mg of the control group; both differences were statistically significant ( $P<0.05$ ). The pregnancy rate of female mice in the experimental group was 60.0%, which was significantly lower than 100.0% in the control group ( $P<0.05$ ). **Conclusion** Long-term exposure to PM2.5 can damage the reproductive function of male BALB/c mice.

**[Key words]** Fine particulate matter 2.5; Mice; Reproductive function; Reproduction toxicity; Oxidative damage

环境细颗粒物(PM2.5)目前被认为是空气污染最主要的有害成份,是全球公共卫生主要挑战之一<sup>[1]</sup>,顾名思义颗粒直径≤2.5 μm,毒性作用与其直径大小存

在密切关系<sup>[2-4]</sup>;此外,PM2.5成份复杂也进一步加重了毒性作用;其表面能够吸附大量有害物质,如有机物多环芳烃、无机物、重金属及致病微生物等,这些有害

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物质深入并沉积在呼吸性细支气管和肺泡中,PM2.5 的直径细小成份甚至可以直接穿过肺间质,进入血液系统,到达全身各个器官发挥毒性作用<sup>[5-6]</sup>。最新研究表明,长期暴露PM2.5不仅促进呼吸系统疾病的发生,并且进入体内可明显增加心血管疾病的发病率和死亡率<sup>[7-9]</sup>,此外,长期吸入PM2.5不仅可诱发糖尿病发生并且能够加重糖尿病的病情<sup>[10-11]</sup>。气管内滴注PM2.5悬浮液或暴露于柴油机尾气显著增加大鼠精子畸形率、减少精子数量和降低雄激素水平<sup>[12]</sup>,但其机制仍不明确,为此本研究以雄性BALB/c小鼠为研究对象,探讨PM2.5对雄性动物生殖功能影响的相关机制。

## 1 材料与方法

### 1.1 实验材料

1.1.1 PM2.5的收集 选取兰州市七里河区西津东路交通繁华路段,使用BGI公司PQ200型PM2.5采样器采集PM2.5细颗粒,具体PM2.5制备方法参考相关文献;样本保存于-20℃冰箱,0.9%氯化钠液制备相应混悬液并灭菌后使用。

1.1.2 动物分组 雄性BALB/c小鼠(40只)购于中国人民解放军联勤保障部队940医院(原兰州总医院)实验动物中心,饲养于中国人民解放军联勤保障部队940医院实验动物中心。严格按照实验动物饲养标准,由专门人员喂养饲料,自由饮水进食。

### 1.2 实验方法

1.2.1 实验动物分组 采用随机数表法将40只BALB/c雄鼠[(20±2)g,28d龄]分为对照组和实验组各20只;对照组给予生理盐水雾化吸入,实验组给予PM2.5混悬液雾化吸入。采用自行设计多功能雾化器,可使密闭容器内充满白雾,并且密闭环境的染毒剂量为(150±10)μg/m<sup>3</sup>,将雄性BALB/c小鼠暴露于密闭容器中,自然染毒,每天染毒6h(9:00~12:00,14:00~17:00),连续暴露12周后,每组随机选取10只BALB/c小鼠,与正常BALB/c雌鼠1:1合笼交配,观察雌鼠受孕情况;剩余小鼠收集附睾、睾丸。

1.2.2 组织病理学检查 小鼠用10%水合氯醛麻醉后,称重、取材,4%多聚甲醛溶液固定睾丸组织,常规石蜡包埋,苏木精-伊红染色,光镜下行病理学检查。

1.2.3 精子计数和畸形率检测 小鼠用10%水合氯醛麻醉后,根据PARHIZKAR等<sup>[13]</sup>介绍的方法,具体操作步骤参考文献。取附睾悬进行涂片,显微镜下观察精子畸形数和精子总数,计算精子畸形率。

1.2.4 抗氧化检测 小鼠右侧睾丸组织用预冷的生理盐水制成10%的匀浆,使用丙二醛(Malonaldehyde,MDA)含量、总抗氧化能力(total antioxidative capacity,TAC)试剂盒(南京建成生物工程有限公司)测定测定睾丸组织中MDA含量、TAC活性,严格按照试剂盒说明书进行测定。

### 1.2.5 雌鼠受孕率 根据合笼的正常BALB/c雌

鼠受孕情况,计算受孕率。

1.3 统计学方法 应用SPSS 19.0软件进行数据分析,计量资料以均数±标准差( $\bar{x}\pm s$ )表示,两两比较采用t检验,两组率的比较采用 $\chi^2$ 检验,检验水准 $\alpha=0.05$ ,以 $P<0.05$ 表示差异有统计学意义。

## 2 结果

2.1 PM2.5对小鼠睾丸组织的影响 睾丸组织HE染色后光镜观察结构改变。对照组小鼠睾丸组织结构完整,少量淋巴细胞浸润(图1A、1B),实验组小鼠睾丸组织炎性细胞浸润明显,生精小管结构发生明显改变,生精细胞层减少,生精细胞结构排列紊乱(图1C、1D)。

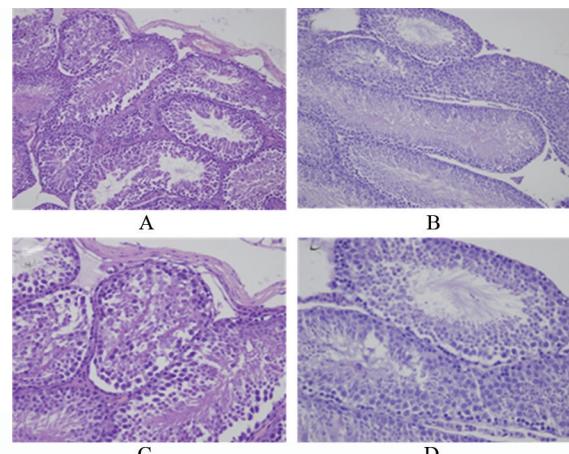


图1 PM2.5对小鼠睾丸组织的影响

注:A、B分别为对照组小鼠睾丸组织的HE染色,视野分别为×200、×400,C、D分别为实验组小鼠睾丸组织的HE染色,视野分别为×200、×400,实验组PM2.5暴露后睾丸组织炎症细胞浸润,睾丸生精小管结构紊乱,管腔中精子数量减少,生精细胞脱落。

2.2 PM2.5对小鼠精液质量的影响 与对照组小鼠相比,实验组小鼠精子总数量即精子浓度明显减少,并且精子畸形率升高,差异均有统计学意义( $P<0.05$ ),见表1。

表1 两组小鼠精子浓度及异常精子数比较( $\bar{x}\pm s$ )

组别	数量	精子浓度( $\times 10^7/mL$ )	畸形率(%)
对照组	10	6.47±1.02	1.56±0.13
实验组	10	4.43±0.55	3.87±0.60
<i>t</i> 值		3.06	-6.58
<i>P</i> 值		<0.05	<0.05

2.3 PM2.5对小鼠睾丸组织抗氧化损伤能力的影响 实验组小鼠睾丸组织中MDA含量与对照组小鼠相比明显增高,而TAC含量明显降低,差异均有统计学意义( $P<0.05$ ),见表2。

表2 两组小鼠睾丸组织氧化损伤指标( $\bar{x}\pm s$ )

组别	数量	TAC (U/mg)	MDA (U/mg)
对照组	10	18.58±2.50	1.53±0.28
实验组	10	13.95±1.34	2.55±0.14
<i>t</i> 值		2.82	-5.63
<i>P</i> 值		<0.05	<0.05

**2.4 PM2.5 对雌鼠受孕率的影响** 对照组、实验组分别有 10 只和 6 只 BALB/c 雌鼠受孕, 受孕率分别为 100.0% 和 60.0%, 差异有统计学意义 ( $\chi^2=4.750, P=0.029$ ); 雄性 BALB/c 小鼠长期暴露 PM2.5 颗粒物, 可能造成雄鼠小鼠生殖能力下降, 最终使雌鼠受孕率下降。

### 3 讨论

不孕不育是人类生殖健康的主要问题, 流行病学研究表明, 世界上 15% 的夫妇没有子女, 其中男性生殖问题占 50%。精子数量和质量的下降在男性不育中起着关键作用。而雄性生殖系统特别容易受到空气污染物的影响<sup>[14-15]</sup>。空气污染物, 尤其是 PM2.5, 一直与精子质量的显著下降有关<sup>[16]</sup>。尽管 PM2.5 环境污染是全球公共卫生最关注的风险因素之一<sup>[17]</sup>, 但阐明其对男性生殖健康机制的研究较少。本实验系统地研究了长期吸入 PM2.5 对雄性 BALB/c 小鼠生殖系统的影响。

本研究采用全身吸入暴露系统模拟真实暴露于空气污染 PM2.5, 因此, 本研究数据为环境 PM2.5 对男性生殖功能的损害提供了更具说服力的证据。在 PM2.5 暴露的小鼠睾丸组织 HE 染色中, 睾丸组织炎症细胞浸润明显, 生精上皮变薄, 生精细胞排列紊乱, 层次减少, 说明 PM2.5 支持小鼠细胞及细胞间的紧密连接, 损伤大鼠睾丸生精功能。精子数量是评价男性生育能力的重要指标之一<sup>[18]</sup>, 本研究通过对附睾精液质量分析, 实验组 PM2.5 混悬液雾化吸入 BALB/c 雄鼠精子数量与对照组生理盐水雾化吸入小鼠相比显著减少, 并且畸形精子数量显著增高。有研究报道大气污染物中细颗粒物能够影响男性精子质量, 产生生殖毒性。另有研究发现环境 PM2.5 水平与精子形态学异常呈正相关, 与精子数量、运动能力和睾丸激素水平呈负相关<sup>[19-20]</sup>, 这与本研究结果相符。综上所述, 这些数据表明环境 PM2.5 暴露导致精子数量下降, 可能归因于精子发生缺陷。

本实验主要研究 PM2.5 对睾丸组织氧化应激损伤, 促进免疫炎症的发生。目前研究发现 PM2.5 能够刺激机体组织产生大量 ROS 和 RNS (活性氮) 物质<sup>[21]</sup>; PM2.5 暴露也可导致机体超氧化物歧化酶(superoxide dismutase, SOD)、过氧化氢酶(Catalase, CAT)活性降低<sup>[22]</sup>, 从而使机体细胞内的 ROS、RNS 升高, 引起机体局部或全身炎症反应, 损害细胞膜脂质、蛋白质、酶及 DNA 等<sup>[23]</sup>, 导致组织细胞生理功能障碍, 甚至引起细胞死亡及引起组织损伤。本实验研究结果表明, 与对照组生理盐水雾化吸入小鼠相比, 实验组 PM2.5 混悬液雾化吸入小鼠睾丸组织中 MDA 含量明显升高, TAC 明显降低, 其中 MDA 是细胞膜氧化损伤的产物。提示 PM2.5 可使得小鼠睾丸组织抗氧化能力减弱, 进而导致生殖细胞损伤。另有研究发现 PM2.5 通过 PI3K/Akt 信号通路造成雄性生殖能力氧化损伤<sup>[24]</sup>。

总之, 本研究结果表明环境中 PM2.5 颗粒可能通

过氧化应激损伤影响雄性小鼠精子数量及质量, 最终损伤雄性 BALB/C 小鼠的生殖功能。

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## 体外共培养模式下小胶质细胞影响内皮细胞紧密连接

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**【摘要】目的** 探讨体外共培养模式下活化的小胶质细胞对人脐静脉内皮细胞(HUVECs)紧密连接的影响。**方法** 原代培养大鼠视网膜小胶质细胞分成4组,分别用不同浓度的脂多糖(LPS)(0 ng/mL、10 ng/mL、100 ng/mL 和 1 000 ng/mL)激活小胶质细胞24 h,通过ELISA检测LPS激活后小胶质细胞分泌肿瘤坏死因子- $\alpha$ (TNF- $\alpha$ )的水平。小胶质细胞接种于Transwell的上室,用含100 ng/mL LPS的DMEM/F12培养24 h后用于实验。共培养体系作如下分组:A组,上室空白,下室HUVECs;B组,上室为未激活的小胶质细胞,下室HUVECs;C组,上室为用100 ng/mL LPS激活24 h的小胶质细胞,下室HUVECs。三组培养体系共培养24 h后,将HUVECs行细胞免疫荧光染色,观察claudin 1的表达,并行Western Blot检测claudin 1和occludin蛋白表达变化。**结果** 四组不同浓度的LPS(0 ng/mL、10 ng/mL、100 ng/mL 和 1 000 ng/mL)激活小胶质细胞24 h后,小胶质细胞分泌TNF- $\alpha$ 的水平不同,分别为(16.36±3.90) pg/mL、(378.46±11.46) pg/mL、(507.11±11.20) pg/mL、(754.55±53.43) pg/mL,组间比较差异有统计学意义( $P<0.05$ )。小胶质细胞和HUVECs共培养24 h后,细胞免疫荧光染色显示C组HUVECs的claudin 1的表达较A组和B组减弱;Western Blot检测共培养体系A组、B组和C组的claudin 1和occludin相对蛋白量,claudin 1蛋白相对表达量在A组、B组和C组分别为0.233±0.010、0.244±0.010、0.171±0.001,occludin蛋白相对表达量在A组、B组和C组分别为0.474±0.045、0.323±0.029、0.139±0.017,三组间比较,claudin 1和occludin的相对蛋白量差异均有统计学意义( $P<0.05$ ),C组的claudin 1和occludin相对蛋白量下调。A组和B组claudin 1的表达差异无统计学意义( $P>0.05$ )。**结论** 活化的小胶质细胞能产生大量的TNF- $\alpha$ ,可能通过上调TNF- $\alpha$ 的表达使HUVECs紧密连接蛋白的表达下调。

**【关键词】** 小胶质细胞;内皮细胞;紧密连接;血-视网膜屏障;肿瘤坏死因子- $\alpha$

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**Effect of microglia on the expression of tight junction in human umbilical vein endothelial cells by co-culture *in vitro*.** LIAO Yu-jie, YU Xiao-yan, JIN Yi-ping, ZHU Hao-hao. Department of Ophthalmology, the Fifth People's Hospital of Shanghai, Fudan University, Shanghai 200240, CHINA

**[Abstract]** **Objective** To investigate the effect of microglia on the expression of tight junction in human umbilical vein endothelial cells (HUVECs) by co-culture *in vitro*. **Methods** Primary microglia of rat was cultured and divided into four groups activated with lipopolysaccharide (LPS) in different concentration for 24 h, including normal control group (0 ng/mL LPS), 10 ng/mL LPS group, 100 ng/mL LPS group, and 1 000 ng/mL LPS group. The protein levels of tumor necrosis factor-alpha (TNF- $\alpha$ ) in the culture media were detected by enzyme linked immunosorbent assay (ELISA). Retinal microglia were cultured onto Transwell permeable support membrane inserts, then activated by lipopolysaccharide (LPS) (100 ng/mL) for 24 h. HUVECs were randomly divided into three groups: HUVECs with empty inserts without cultured microglia (group A), HUVECs with untreated microglia (group B), and HUVECs with 100 ng/mL

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