臭氧急性暴露对大鼠血管的损伤及其机制*

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【摘要】 目的：探索不同浓度臭氧(O_3)急性暴露对雄性 Wistar 大鼠血管的损伤效应和可能的机制。方法：120 只雄性 Wistar 大鼠随机分为 6 组，每组 20 只；实验动物置于气体染毒柜中，对照组暴露于过滤后空气，处理组分别暴露于浓度为 0.12 ppm，0.5 ppm，1.0 ppm，2.0 ppm 和 4.0 ppm 的臭氧，持续暴露 4 h。利用 PC-lab 医学生理信号采集系统获得动脉血压数据；血流变指标和血生化指标由天津迪安诊断实验室检测；血清中内皮素(ET-1)、同型半胱氨酸(HCY)、血管性血友病因子(vWF)、8-羟基脱氧鸟苷(8-OhdG)、白介素(IL-6)和肿瘤坏死因子 α(TNF-α)采用酶联免疫(ELISA)微孔板法检测；氧化应激指标超氧化物歧化酶(SOD)活力和丙二醛(MDA)分别采用黄嘌呤氧化酶法、硫代巴比妥酸(TBA)法测定，还原型谷胱甘肽(GSH)和一氧化氮(NO)采用微孔板比色法；取胸主动脉组织制备石蜡切片，经 HE 染色后观察血管结构改变。结果：0.12 ppm 臭氧急性暴露可导致动脉收缩血压(SBP)显著升高；不同浓度臭氧暴露均可导致血浆粘度显著升高，1.0 ppm 臭氧暴露组血沉(ESR)方程 K 值显著升高，全血高切相对指数和还原粘度均在臭氧浓度为 0.5 ppm 和 4.0 ppm 时显著降低，而红细胞变形指数在臭氧浓度为 0.12 ppm、0.5 ppm、1.0 ppm 和 2.0 ppm 时显著升高；急性臭氧暴露可导致总胆固醇含量降低，高密度脂蛋白胆固醇(HDL-C)在 0.12 ppm 臭氧暴露组显著降低；当臭氧浓度高于 1.0 ppm 时还可导致机体出现炎症反应(TNF-α 升高)和氧化应激反应(MDA 升高、GSH 降低)；臭氧急性暴露可导致血液中 ET-1 含量升高，在 4.0 ppm 浓度组具有显著性差异，而 HCY 水平呈现先降低后升高的趋势，在 1.0 ppm 浓度组达到最高值，胸主动脉未见明显的病理改变。结论：臭氧急性暴露可影响大鼠的动脉血压、血流变及胆固醇代谢，可能的机制是臭氧暴露导致炎症反应和氧化应激反应，引起血管内皮功能损伤，并且随着臭氧暴露浓度升高血管内皮细胞功能损伤越显著。

【关键词】 臭氧；大鼠；血管损伤；炎症因子；血流变；氧化应激；血脂
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Injury of rat blood vessels caused by acute ozone exposure and its mechanism

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【ABSTRACT】**Objective:** To investigate the vascular damage effects and possible mechanism of acute exposure to ozone (O_3) in male Wistar rats. **Methods:** One hundred and twenty male Wistar rats were randomly divided into six groups, 20 in each group. The experimental animals were placed in a gas poisoning cabinet, the control group was exposed to filtered air, and the treatment group was exposed to ozone at concentrations of 0.12 ppm, 0.5 ppm, 1.0 ppm, 2.0 ppm, and 4.0 ppm, respectively, for 4 hours. Arterial blood pressure data were obtained by PC-lab medical physiological signal acquisition system. Blood rheology indicators and blood biochemical indicators were detected by Tianjin Dean Diagnostic Laboratory. Serum endothelin-1 (ET-1), homocysteine (HCY), von Willebrand factor (vWF), 8-hydroxydeoxyguanosine (8-OhdG), interleukin (IL-6) and tumor necrosis factor alpha (TNF-α) were detected by enzyme-linked immunosorbent assay (ELISA) microplate assay. Oxidative stress indicators superoxide dismutase (SOD) activity and malondialdehyde (MDA) were determined by xanthine oxidase method, thiobarbituric acid (TBA) method, reduced glutathione (GSH) and nitric oxide (NO) were tested by using microplate colorimetry. Paraffin sections were prepared from thoracic aorta tissue, and vascular structure was observed by HE staining. **Results:** Acute exposure to 0.12 ppm ozone could cause a significant increase in arterial systolic blood pressure (SBP). Exposure to different concentrations of ozone could cause a significant increase in plasma viscosity, and the K value of the ESR equation was significantly increased in the 1.0 ppm ozone exposure group. Both the relative and reduced viscosities were significantly reduced at ozone concentrations of 0.5 ppm and 4.0 ppm, while the red blood cell deformation index was increased significantly at ozone concentrations of 0.12 ppm, 0.5 ppm, 1.0 ppm, and 2.0 ppm. Acute ozone exposure resulted in the de-

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crease of total cholesterol content. The content of high-density lipoprotein cholesterol (HDL-C) was significantly reduced in the 0.12 ppm ozone exposure group. When the ozone concentration was higher than 1.0 ppm, the body may also had an inflammatory reaction (increased TNF-α) and oxidative stress (increased MDA, decreased GSH). Acute exposure to ozone could lead to elevated levels of ET-1 in the blood, with significant differences in the 4.0 ppm concentration group, while HCY levels were decreased firstly and then increased, reaching the highest in the 1.0 ppm concentration group. No obvious pathological changes were observed in the thoracic aorta. **Conclusion:** Acute ozone exposure can affect arterial blood pressure, blood rheology and cholesterol metabolism in rats. The possible mechanism is that ozone exposure leads to inflammatory reaction and oxidative stress reaction, causing vascular endothelial function damage, and vascular endothelial cells increase with ozone exposure concentration.

【KEY WORDS】 ozone; rat; vascular injury; inflammatory factor; blood rheology; oxidative stress

随着我国经济的飞速发展,空气污染问题日益突出。除了广泛关注的固态颗粒物(particulate matter,PM)外,臭氧(O_3)越来越成为夏季重要的环境污染物,臭氧与人群健康的关系也逐步受到重视,已成为研究的热点。臭氧是一种强氧化性的气体,一方面可与直接接触的人体组织(呼吸道、肺、角膜等)产生强烈的氧化效应,导致组织损伤[1];另一方面,可通过与肺泡液及肺泡上皮细胞反应生成氧化产物(醛类,羧酸和过氧化氢),产物随后进入血液循环,对机体产生更广泛的损伤[2]。血管在机体内参与多种生理生化过程,包括物质运输、维持调节血压、参与凝血及抗凝反应,炎症反应以及内分泌功能。血管损伤是动脉粥样硬化形成、血栓性疾病的重要原因。流行病学研究已显示臭氧暴露会增加心血管疾病死亡率。在中国,大气臭氧浓度每增加 10 μg/m^3,人群中心血管疾病死亡率增加 0.448%(95% CI 0.171%,0.724%)[3,4]。进一步研究发现,短期臭氧暴露导致心血管死亡率增加的可能机制是血压升高和血小板活化[5],或者通过颗粒物来间接介导损伤[6]。而 Xie 等[7]研究发现,大鼠腹腔注射的臭氧可以导致炎症因子和血管内皮生长因子升高,这提示臭氧可以导致炎症并促进血管内皮增生。Miller 等[8]的研究发现,臭氧暴露后可增加胚胎植入后的 Long-Evans 大鼠子宫动脉血流阻力增加。Micheal 等[9]的实验发现臭氧 0.8 ppm 持续 24 h 急性暴露后,SD 大鼠离体冠脉血管对乙酰胆碱碱的逆收缩反应降低。目前有关臭氧单因素多浓度急性暴露对血管损伤和机制的研究尚未见报道,因此,本研究选择不同浓度臭氧建立大鼠急性暴露模型,观察其对血管的结构与功能,对血液中多种生化、代谢指标,以及氧化应激和炎症反应等的影响,明确臭氧急性暴露对血管的损伤效应,并探讨可能的作用机制,为防治臭氧暴露导致的心血管系统损伤提供科学依据。

1 材料与方法

1.1 材料

气体染毒柜(HRH CSED-K,北京慧荣和),臭氧发生器(FW-120,Vonti,中国),臭氧监测仪(1003 AH,CA,Glendale),医学生理信号采集系统(PClab-UE,北京微信斯达科技有限公司),冷冻离心机(TGL-20M,湘仪),连续可见光谱酶标仪(xMark Microplate Spectrophotometer,Bio-Rad),液相芯片仪(Luminex200,德国 Merk 公司),血生化分析仪(C501,罗氏),血流变分析仪(ZL6000,众驰伟业);Wistar 大鼠(北京维通利华实验动物有限公司),大鼠内皮素 1(endothelin-1,ET-1)、血管性血友病因子(von willebrand factor,vWF)、同型半胱氨酸(homocysteine,HCY)、白介素 6(interleukin-6,IL-6)和肿瘤坏死因子 α(tumor necrosis factor-α,TNF-α)检测试剂盒酶联免疫吸附(ELISA)试剂盒(北京博奥拓达),丙二醛(malondialdehyde,MDA)、谷胱甘肽(glutathione,GSH)、超氧化物歧化酶(superoxide dismutase,SOD)、一氧化氮(nitric oxide,NO)检测试剂盒(南京建成生物科技公司)。10%甲醛固定液、无水乙醇、二甲苯和苏木素-伊红(HE)染液(Solarbio 公司)。

1.2 动物分组与处理

健康雄性 Wistar 大鼠 120 只,体重 175~200 g,随机分为 6 组,每组 20 只,饲养环境为温度(21±1)℃,湿度 50%±5%,12 h 的光照/黑夜循环。适应性喂养 5 d 后,将大鼠放入气体染毒柜中,对照组暴露于过滤后空气,处理组分别暴露于浓度为 0.12 ppm、0.5 ppm、1.0 ppm、2.0 ppm 和 4.0 ppm 的臭氧,持续 4 h。暴露期间气体染毒柜中的温度保持在 22.6~25.6℃,湿度保持在 35%~65%。暴露结束后,每组取 10 只大鼠,称重后,用 3%戊巴比妥钠(40 mg/

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