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• 裸鼹鼠研究专题 •

脂多糖对裸鼹鼠肺脏组织影响的初探

张成财[1*]，刘尹航[2*]，陈 超[1]，江文正[3]

（1. 海军军医大学基础医学院实验动物学教研室，上海 200433;
2. 上海外国语大学附属中学，上海 200083; 3. 华东师范大学生命科学学院，上海 200241）

[摘要] **目的** 观察和分析脂多糖（lipopolysaccharide，LPS）对裸鼹鼠肺脏组织形态结构和病理学相关指标的影响，初步探讨裸鼹鼠是否存在LPS耐受。**方法** 雄性裸鼹鼠10只，随机分为实验组和对照组。实验组按体质量以10 mg/kg给药量腹腔注射LPS，对照组注射同等体积的0.9%NaCl溶液（生理盐水）。给药后6 h，采集肺脏，检测肺组织湿干重量比值；制作石蜡切片，经HE染色后，光学显微镜下观察肺组织显微结构；流式细胞仪检测肺组织细胞中活性氧含量、肺组织细胞凋亡率。**结果** 实验组裸鼹鼠肺组织结构完整，肺泡孔内无明显渗出液和炎性细胞浸润等病变。与对照组比较，实验组肺组织湿干质量比未发生显著变化，早期凋亡率未发生显著变化，但晚期凋亡率显著升高（$P < 0.01$），活性氧含量显著升高（$P < 0.05$）。**结论** 裸鼹鼠对LPS诱导的肺损伤有一定程度的耐受性，其具体机制有待进一步研究。

[关键词] 裸鼹鼠；肺脏；脂多糖

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裸鼹鼠属于哺乳纲啮齿目裸鼹鼠属裸鼹鼠种，原产于非洲，主要以植物的根茎为食[1-2]。裸鼹鼠长期生活在地下2 m左右的洞穴中，依靠身体上的触毛感受周围的环境。裸鼹鼠因具有对癌症的免疫能力等生物学特性，近年来越来越受到科研工作者关注[3]。

急性肺损伤和急性呼吸窘迫综合征是除心源性以外的各种肺内外致病因素所致的急性进行性呼吸功能衰竭[4]，多见于严重感染、休克、创伤等疾病的发生过程中，表现为以中性粒细胞为主的肺泡炎性细胞浸润、肺毛细血管内皮和肺泡上皮损伤导致的弥漫性肺水肿[5-6]。尽管现代医疗水平已经取得长足进步，然而急性呼吸窘迫综合征的病死率仍高达40%～68.5%[7-9]。因此，寻找更加有效的急性肺损伤治疗方法具有重大意义。本实验初步探讨腹腔注射脂多糖（lipopolysaccharide，LPS）对裸鼹鼠肺脏组织的影响，为进一步研究其机制奠定基础。

1 材料与方法

1.1 实验动物

12月龄健康雄性裸鼹鼠10只，由海军军医大学实验动物中心生产，饲养于屏障设施[SYXK（沪）2017-0004]。

1.2 实验动物分组与实验处理

10只裸鼹鼠随机分为对照组5只和实验组5只。实验前12 h裸鼹鼠禁食不禁水，称质量后实验组按体质量以10 mg/kg给药量腹腔注射LPS，对照组注射等体积的0.9%NaCl溶液（生

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[作者简介] 张成财(1992—), 男, 硕士研究生。研究方向: 动物学。E-mail: zhangchengcai_06@163.com
刘尹航(2002—), 男, 学生。
E-mail: liuyinhang_27@163.com
*共同第一作者

[通信作者] 江文正(1973—), 男, 博士, 教授。研究方向: 免疫学。E-mail: wzjiang@bio.ecnu.edu.cn

理盐水）。

1.3 主要仪器设备及试剂

流式细胞仪购自美国 Beckeman 公司；烘箱购自上海精宏实验设备有限公司；自动组织脱水机、包埋机、转轮式切片机、染色机、封片机和倒置荧光显微镜等均购自徕卡显微系统（上海）贸易有限公司；活性氧检测试剂盒购自碧云天生物技术研究所；凋亡检测试剂盒购自美国 BD 公司；LPS 购自美国 Sigma 公司。

1.4 实验方法

腹腔注射 LPS 6 h 后，按体质量以 50 mg/kg 给药量，腹腔注射 0.375% 戊巴比妥钠，麻醉处死裸鼹鼠。

1.4.1 *肺脏组织切片的制备与观察* 打开胸腔，取肺脏右侧下叶，置于通用型中性组织固定液中固定 24 h。经脱水、透明、包埋、切片、染色、封固等步骤完成 HE 染色组织切片的制备，光学显微镜下观察。

1.4.2 *肺组织湿干重比的测定* 打开胸腔，取出肺脏左侧。PBS 缓冲液冲洗干净，并用滤纸吸干。左肺置于电子天平称质量，计作湿质量。置于烘箱（80 ℃，48 h）烤至质量恒定，称量后计为干质量。计算肺湿干质量比（= 湿质量 / 干质量）。

1.4.3 *肺组织细胞活性氧含量与凋亡检测* 取右侧肺脏上叶和中叶，在超净台研磨提取细胞。用直径为 70 μm 的细胞筛滤过后，加入红细胞裂解液去除红细胞，调整细胞密度为 1×10^6/mL。按照说明书方法添加检测试剂，流式细胞仪检测肺组织细胞活性氧含量（以平均荧光值表示）和凋亡率。

1.5 统计学处理

采用 SPSS17.0 统计软件进行分析处理。数据以 $\bar{x} \pm s$ 表示，组间比较采用 t 检验，$P < 0.05$ 为差异有统计学意义。

2 结果

2.1 LPS 对裸鼹鼠肺组织湿干质量比的影响

与对照组（n=5）肺组织湿干质量比（5.10 ± 0.20）比较，实验组（n=5）肺组织湿干质量比（5.04 ± 0.14）未发生显著变化（$P < 0.05$）。

2.2 LPS 对裸鼹鼠肺组织细胞氧化应激的影响

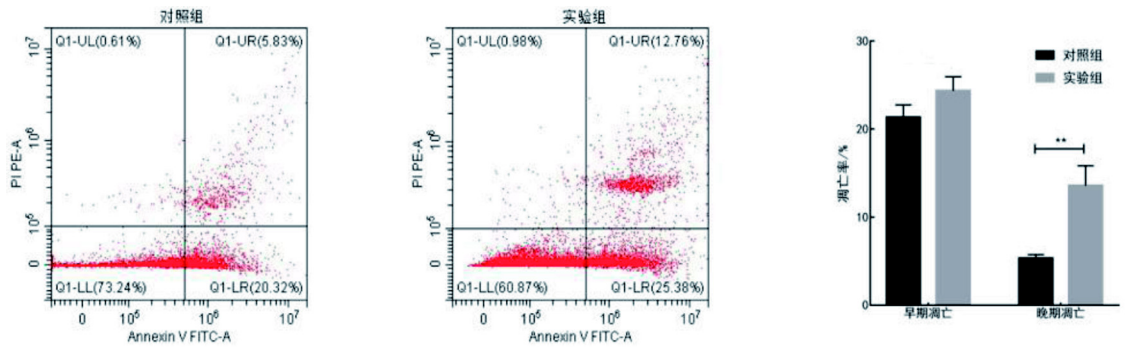
与对照组（n=5）肺组织细胞活性氧含量（19 913 ± 4 700）比较，实验组（n=5）肺组织细胞活性氧含量（35 325 ± 2 989）显著升高（$P < 0.05$）。

2.3 LPS 对裸鼹鼠肺组织细胞凋亡率的影响

流式细胞仪检测显示，与对照组比较，实验组肺组织细胞早期凋亡率未发生明显变化，晚期凋亡率显著升高（$P < 0.01$，图 1）。

2.4 LPS 对裸鼹鼠肺组织结构的影响

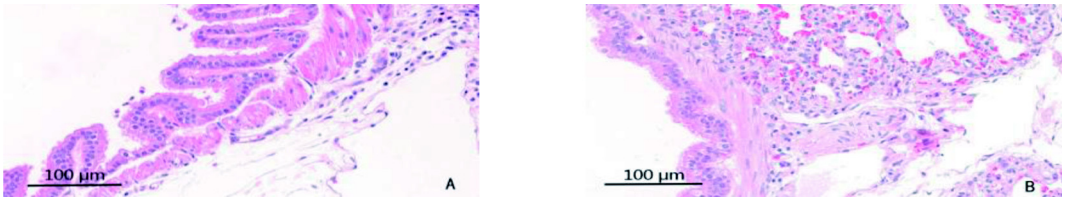
光学显微镜观察显示，对照组（图 2A）和实验组（图 2B）肺组织结构完整，肺泡孔内无明显渗出液和炎性细胞浸润。



与对照组比较，$^*P < 0.05$，$^{**}P < 0.01$；n=5。

图 1 裸鼹鼠肺组织细胞凋亡率

Figure 1 Apoptosis rate of lung tissue cells in naked mole rats



A 为对照组，B 为实验组

图 2 裸鼹鼠肺组织病理学观察

Figure 2 Pathological observation on lung tissues in naked mole rats

3 讨论

目前临床常见的感染以革兰阴性杆菌多见，其主要致病原是内毒素（endotoxin，ET），而 LPS 是内毒素的主要成分，故现在已不再区分 LPS 和 ET[10-11]。有研究表明，腹腔注射一定剂量的 LPS 能够引起急性肺损伤[12]。急性肺损伤大致可以分为急性渗出期、亚急性纤维增殖期和慢性纤维化应答期[13]。急性渗出期主要表现为高通透性肺泡水肿、出血和血栓形成，影响通气血流比值和肺泡换气，表现为急性低氧血症[14-15]。主要病理改变为炎性反应和凝血反应，导致肺微血管内皮细胞和上皮细胞损伤，以及肺泡毛细血管膜屏障功能完整性的破坏[16]。机体在受到 LPS 刺激时，先产生非特异性免疫应答，主要通过循环和组织炎性细胞介导完成。这些炎性细胞在正常时没有活性，当受到病原刺激时，可以被快速激活，并通过产生炎性介质和活性氧等引起组织损伤[17]。

裸鼹鼠在自然状态下生活于封闭的地下洞道，不易与外界进行气体交换，致使洞道内长期维持低氧及高二氧化碳的气体环境[18-19]，在长期进化过程中，裸鼹鼠已经产生了一系列生理改变以适应这种气体环境。本实验给裸鼹鼠腹腔注射 LPS，肺组织湿干质量比未发生显著变化，表明裸鼹鼠并没有发生急性肺损伤，病理学观察也验证了此结论；裸鼹鼠肺组织内活性氧含量显著升高，但是肺组织并未受到损伤，提示裸鼹鼠体内可能存在未发现的抗损伤机制；肺组织细胞晚期凋亡率显著升高，但是早期凋亡比例没有发生显著变化，这可能是由于裸鼹鼠肺脏组织中存在强大的细胞储备，在机体遭受损伤时，能够快速动员储备细胞，维持机体健康。

综上所述，虽然受 LPS 干预的裸鼹鼠肺脏组织中活性氧含量与晚期凋亡率显著升高，但是肺组织湿干质量比和肺组织细胞早期凋亡率并未发生显著变化，病理学观察也未见相应病变。结果表明，裸鼹鼠对 LPS 诱导的急性肺损伤有一定程度耐受，其具体机制有待进一步研究。

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A Preliminary Study on Effects of Lipopolysaccharide on Lung Tissues of Naked Mole Rats

ZHANG Chengcai[1*], LIU Yinhang[2*], CHEN Chao[1], JIANG Wenzheng[3]

(1. Department of Laboratory Animal Science, School of Basic Medical Sciences, Naval Medical University, Shanghai 200433, China; 2. Shanghai Foreign Language School, Shanghai 200083, China; 3. School of Life Sciences, East China Normal University, Shanghai 200241)

[*]These two authors contributed equally.

Correspondence to: JIANG Wenzheng, E-mail: wzjiang@bio.ecnu.edu.cn

[Abstract] Objective To detect the effects of lipopolysaccharide (LPS) on lung morphology and pathological parameters, and explore the LPS tolerance in naked mole rats. **Methods** Ten male naked mole rats were randomly divided into an experimental group and a control group. The naked mole rats of the experimental group were injected with LPS at 10 mg/kg according the body weight, while the naked mole rats of the control group were injected with the same volume of normal saline. Six hours after the administration, lung tissues were collected to detect the ratio of wet to dry weight; paraffin sections were prepared and stained with HE, and the lung microstructure was observed under optical microscope; the level of reactive oxygen species (ROS) and the apoptosis ratio of lung tissue cells were

detected by flow cytometry. **Results** Microscopic examination showed that the lung tissue structure of naked mole rats in the experimental group was intact, and there were no obvious exudate and inflammatory cell infiltration in the alveolar foramen. Compared with the control group, the ratio of wet to dry weight of lung tissues in the experimental group and the proportion of early apoptosis had no significant differences, but the proportion of late apoptosis and the content of ROS increased significantly ($P < 0.01$, and $P < 0.05$, respectively). **Conclusion** Naked mole rats can tolerate the lung injury induced by LPS to some degree, and the specific mechanism needs to be further studied.
[Key words] Naked mole rats; Lung; Lipopolysaccharide

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Effect of ^{60}Co γ-ray Radiation on Spleen of Naked Mole Rats

YANG Rong[1], ZHAO Yining[2], YANG Wenjing[1], ZHAO Shanming[1],
CHAI Yujing[2], ZHANG Chengcai[1], YUAN Zheng[3#], CUI Shufang[1#]
(1. Department of Laboratory Animal Science, School of Basic Medical Sciences, Naval Medical University, Shanghai 200433, China; 2. Department of Medical Technology, Yangpu District Mental Health Center, Shanghai 200090, China; 3. Laboratory Animal Center, Academy of Military Medical Sciences, Beijing 100071, China)

[#]Correspondence to: CUI Shufang, E-mail: youngstar_sf@163.com
YUAN Zheng, E-mail: yuanzheng001@126.com

[Abstract] Objective To observe the changes of immune system and tissue biochemical indexes in the spleen of naked mole rats exposed to ^{60}Co γ-ray radiation, for probing the radiation tolerance characteristics of the spleen of naked mole rats. **Methods** The whole body of naked mole rats was irradiated with a radiation dose rate of 1.163 Gy/min and a total dose of 10 Gy ^{60}Co γ-rays. The spleens of the normal control and the exposed naked mole rats after 7 d, 14 d, and 21 d of irradiation were collected. The proportions of B lymphocytes and macrophages in the spleens were measured by flow cytometry. Malondialdehyde (MDA), total antioxidative capacity (T-AOC), glutathione (GSH) and oxidative glutathione (GSSG) in the spleen tissues were measured with corresponding biochemical assay kits. **Results** Flow cytometry results showed that the numbers of both B lymphocyte and macrophages increased significantly after irradiation ($P<0.001$). Biochemical examination results showed that the MDA level in spleen was significantly increased ($P<0.05$) after irradiation, the GSH and GSSH levels were significantly decreased ($P<0.05$), the T-AOC level was first significantly decreased ($P<0.01$) and then raised 21 days after irradiation, which was significantly higher than the indexes 7 days and 14 days after irradiation ($P<0.01$). **Conclusion** The spleens of naked mole rats can tolerate ^{60}Co γ-ray radiation to some extent, but the mechanism of tolerance is not clear.
[Key words] Naked mole rats; Radiation tolerance; Spleen; Immunology; ^{60}Co γ-rays