新生大鼠高氧性肺损伤肺组织内源性谷氨酸释放的变化

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摘要: 目的 探讨谷氨酸在新生大鼠高氧性肺损伤中的作用。**方法** SD 新生大鼠,出生后 12 h 内随机分为空气对照组和高氧组。高氧组维持氧浓度≥95%,分别在 1,3 和 7 d 后每组处死 5 只大鼠,取肺脏,测定肺组织湿重/干重(W/D)比值,HE 染色观察肺组织病理变化;另取小鼠,进行支气管肺泡灌洗,制备支气管肺泡灌洗液(BALF),用血细胞计数板进行白细胞计数,全自动生化分析仪测定乳酸脱氢酶(LDH)活性,Lowry 法检测总蛋白含量,高效液相色谱法检测谷氨酸含量。**结果** 与空气对照组比较,持续高氧暴露 1 d 新生大鼠肺组织 W/D 比值无明显变化,暴露 3 和 7 d W/D 比值明显增加。HE 染色可见,持续高氧暴露 3 d 肺泡腔内少量炎症细胞渗出,暴露 7 d 肺泡内红细胞和炎症细胞进一步增多,肺组织结构紊乱,肺泡数量减少。持续高氧暴露 1 d 新生大鼠 BALF 中 LDH 活性明显增加,白细胞计数和总蛋白含量无明显变化,暴露 3 和 7 d BALF 中 LDH 活性、总蛋白含量和白细胞计数均高于空气对照组。持续高氧暴露 1 和 3 d BALF 中谷氨酸含量亦明显高于空气对照组。**结论** 高浓度氧可引起新生大鼠急性肺损伤,诱导肺组织内源性谷氨酸的释放,提示谷氨酸在高氧性肺损伤中发挥重要作用。

关键词: 高氧症; 急性肺损伤; 谷氨酸盐类

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谷氨酸是中枢神经系统重要的兴奋性神经递质。谷氨酸 *N*-甲基-*D*-天冬氨酸(*N*-methyl-*D*-aspartate, NMDA)受体的过度激活可引起神经细胞的急性肿胀、迟发性坏死和凋亡。研究表明,腹腔注射谷氨酸 NMDA 受体阻滞剂地佐环平(dizocilpine, MK-801)可明显减轻高氧暴露 7 d 后新生大鼠肺损伤,表明谷氨酸可能通过 NMDA 受体在高氧性肺损伤的发生发展中发挥重要作用[1],但急性肺损伤时是否存在谷氨酸的过量释放尚未见报道。本研究在观察持续高氧暴露致肺损伤逐渐加重的基础上,观察高氧暴露不同时间新生大鼠肺组织内源性谷氨酸释放的变化,进一步阐明谷氨酸在新生大鼠高氧性肺损伤中的重要作用。

1 材料与方法

1.1 模型制备及分组

孕 22 d 顺产 SD 新生大鼠,清洁级,体重 6 ~ 10 g,中南大学湘雅医学院实验动物中心提供,动物许可证号 SCXK(湘)2006-0002,雌雄兼用。出生后 12 h 内编号并随机分为空气对照组和高氧模型组。模型制备参考文献[2],将高氧组大鼠置于有机玻璃箱内,持续输入氧气,维持氧浓度≥95%,每天用 CYS-1 数字式测氧仪(上海嘉定学联仪表厂)监测 3 次;用钠石灰吸收 CO_2,使 CO_2 浓度≤5%。空气对照组置于同一室内,接受常压空气。箱内及室内温度为 25 ~ 26℃,湿度60% ~70%(无水氯化钙吸收水蒸气)。每天定时开箱 5 min,记录 2 组存活动物数,观察一般情况,添加水及饲料,更换垫料,并与空气对照组互换母鼠。分别在高氧暴露 1,3 和 7 d 时每组随机抽取 5 只大鼠处死进行肺湿重/干重(wet-to-dry weight, W/D)比值测定及肺组织病理学检查,每组随机抽取 6 ~ 11 只进行支气管肺泡灌洗(bron-

choalveolar lavage fluid, BALF)及相关指标检测。

1.2 肺湿重/干重比值测定

新生大鼠腹主动脉放血处死后,直接剪开胸腔,暴露肺组织,小心分离气管及肺脏,结扎右支气管,从结扎的右支气管取下右肺,清除非肺组织,轻轻擦干表面,立即称重,记为肺湿重,然后置于80℃烤箱内,每天称重直至连续2 d无明显变化,记为肺干重,计算肺W/D比值。

1.3 肺组织病理学检查

大鼠处死后,直接打开胸腔结扎右支气管,气管插管,经导管向左肺以2.26 kPa的压力[3]缓缓注入4%甲醛至肺尖膨胀,固定20 min后取下左肺浸泡于4 %甲醛固定,顺序脱水,透明,浸蜡,包埋成蜡块,常规制备石蜡切片,HE染色,光镜下观察肺组织病理变化。

1.4 BALF收集及相关指标检测

给予戊巴比妥钠(90 mg·kg^{-1}, ip)处死大鼠,打开腹腔切断腹主动脉进一步放血处死。颈正中暴露气管,剪开一小口,插入自制导管并固定,由导管向肺内缓缓注入冷生理盐水(35 mL·kg^{-1} ×3 次)[3],然后轻轻回抽,BALF回收率为80% ~90 % 。血液污染或回收率低于70%以下的BALF弃去。采集BALF后,取10 μL在光学显微镜下用血细胞计数板进行白细胞计数。将BALF离心取上清液,用全自动生化分析仪(日立-7170型)测定乳酸脱氢酶(lactate dehydrogenase ,LDH)活性,Lowry等[4]法检测总蛋白含量,高效液相色谱仪(LC-10DVP型,日本岛津)检测谷氨酸含量。

1.5 数据以 $\bar{x} \pm s$ 表示,用SPSS15.0统计软件采用完全随机设计的单因素方差分析及SNK多重比较进行统计学分析。

2 结果

2.1 持续高氧暴露不同时间新生大鼠肺组织湿重/干重比值的变化

与空气对照组比较,持续高氧暴露1 d,新生大鼠肺组织W/D比值无明显变化;持续高氧暴露3和7 d后,W/D比值明显增加,表明高氧组新生大鼠肺水肿程度明显高于空气对照组(表1)。

2.2 持续高氧暴露不同时间新生大鼠肺组织的病理变化

空气对照组新生大鼠出生后第1天肺泡结构不规则,肺泡间隔较宽(图1A);第3天时,肺泡间隔增多并变薄(图1B),第7天时肺泡结构规则且分布均匀(图1C),表明大鼠出生后肺泡结构发育逐渐成熟。与空气对照组比较,高氧组新生大鼠持续高氧暴露1 d肺泡结构无明显变化(图1D),暴露3 d肺泡腔内可见少量炎症细胞(图1E),暴露7 d肺泡内红细胞和炎症细胞进一步增多,肺组织结构紊乱,肺泡数量减少(图1F)。提示持续高氧暴露可导致新生大鼠急性肺损伤,并影响肺泡的正常发育。

Tab 1. Changes in wet-to-dry weight ratio (W/D) of lung tissue from newborn rats after hyperoxia exposure

Group	W/D		
	1	3	7(d)
Air control	5.42 ± 0.05	5.95 ± 0.27	5.65 ± 0.18
Hyperoxia	5.47 ± 0.21	6.14 ± 0.26$^{*\#}$	6.66 ± 0.12$^{**}_{\#\#\triangle}$

The newborn rats in hyperoxia group were exposed to above 95% oxygen for 1, 3 and 7 d, respectively. Rats in air control group were placed in in-door air condition. $\bar{x} \pm s$, $n = 5$. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with air control group on the same day; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with 1 d on the same group; $^{\triangle}P < 0.05$, compared with 3 d on the same group.

2.3 持续高氧暴露不同时间新生大鼠BALF中LDH活性、白细胞总数和总蛋白含量的变化

高氧暴露1 d,高氧组新生大鼠BALF中LDH活性明显增加,白细胞总数和总蛋白含量无明显变化;随着高氧暴露时间延长,在3 d和7 d时肺泡内细胞总数和总蛋白含量逐渐增高,LDH活性逐渐下降,但仍明显高于空气对照组,表明高氧暴露的早期以肺细胞损伤为主,随着高氧暴露时间的延长,肺泡-毛细血管膜屏障受损和炎性渗出加重(表2)。

2.4 持续高氧暴露不同时间新生大鼠BALF中谷氨酸含量的变化

正常新生大鼠出生1 d后,BALF中谷氨酸含量较高,出生后3 d谷氨酸含量迅速下降,至出生后7 d与出生后3 d比较无明显降低,提示正常情况下,肺内存在一定量的谷氨酸释放。高氧可使BALF中谷氨酸含量明显增加,在持续高氧暴露1和3 d BALF中谷氨酸含量明显高于空气对照组,表明高氧可促使肺组织内源性谷氨酸释放增加。但高氧暴露7 d时,BALF中谷氨酸含量低于空气对照组(表2)。

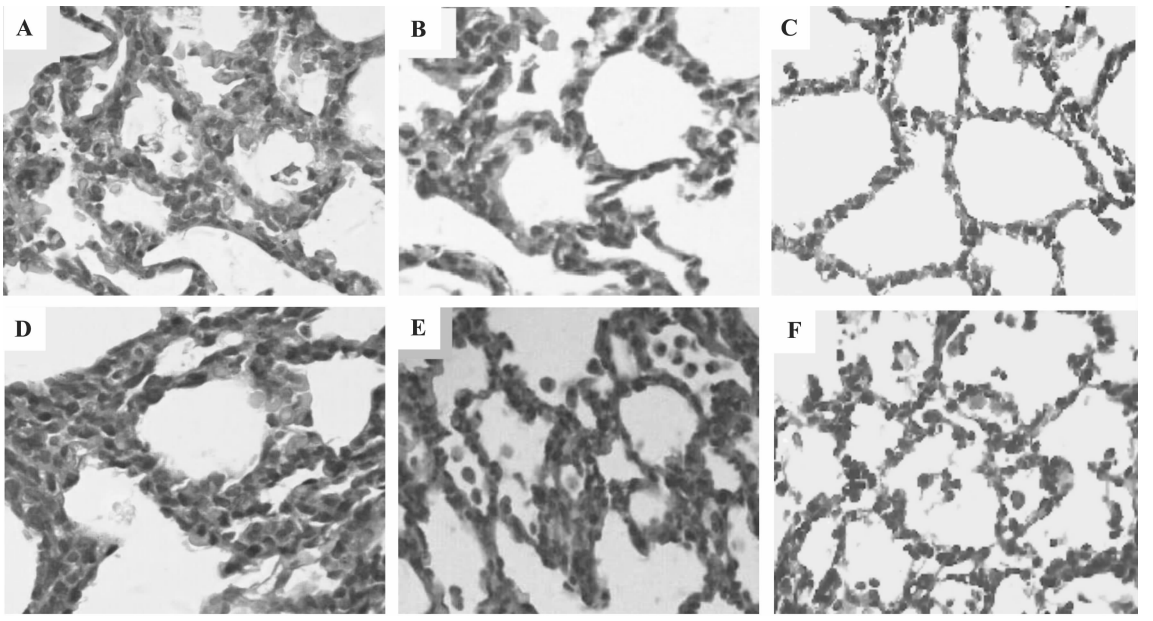

Fig 1.　Lung histopathological changes in newborn rats after hyperoxia exposure (HE staining, × 400).
See Tab 1 for the rat treatments. A, B and C: air control group for 1, 3 and 7 d, respectively; D, E and F: hyperoxia group for 1, 3 and 7 d, respectively.

Tab 2.　Changes in lactate dehydrogenase (LDH) activity, leucocyte count, total protein and glutamate contents in bronchoalveolar lavage fluid of newborn rats after hyperoxia exposure

Group	Exposure time/d	*n*	LDH/U·L^{-1}	Leucocytes count /10^7 L^{-1}	Total protein /mg·L^{-1}	Glutamate /μmol·L^{-1}
Air control	1	11	4.1 ± 2.1	28 ± 8	5.6 ± 2.3	8.3 ± 1.8
	3	6	3.9 ± 1.7	25 ± 3	11.4 ± 1.9$^{\#}$	2.8 ± 1.0$^{\#\#}$
	7	8	3.7 ± 1.4	36 ± 12	14.4 ± 5.2$^{\#\#}$	2.2 ± 0.4$^{\#\#}$
Hyperoxia	1	8	68.0 ± 16.0**	38 ± 7	6.3 ± 0.7	12.4 ± 1.4**
	3	8	38.7 ± 10.6$^{**\#\#}$	95 ± 6$^{**\#\#}$	21.8 ± 5.8$^{*\#\#}$	4.7 ± 0.8$^{**\#\#}$
	7	9	29.6 ± 2.4$^{**\#\#\triangle}$	379 ± 69$^{**\#\#\triangle\triangle}$	196.1 ± 35.5$^{**\#\#\triangle\triangle}$	1.2 ± 0.3$^{**\#\#\triangle}$

See Tab 1 for the rat treatments. $\bar{x} \pm s$. $^{*}P<0.05$, $^{**}P<0.01$, compared with air control group on the same day; $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with 1 d on the same group; $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$, compared with 3 d on the same group.

3　讨论

临床上长期吸入高浓度氧气是引起新生儿肺损伤的常见问题,严重者可以引起支气管肺发育不良[5]。据报道,新生大鼠在 95% 高浓度氧持续暴露 3 d 后即出现肺泡腔内炎症细胞的聚集[6],7 d 后肺泡炎症反应及肺水肿更为明显[2]。本研究结果表明,新生大鼠持续吸入 95% 高浓度氧气 3 d 肺组织 W/D 比值明显增加;肺组织形态学观察也发现,高氧暴露 3 d 肺泡腔内可见少量炎症细胞;7 d 上述变化更为明显。由此表明,新生大鼠高氧性肺损伤模型制备成功[2,6]。另外,检测了 BALF 中 LDH 活性、总蛋白含量及白细胞数。LDH 存在细胞内,BALF 中 LDH 活性的增高常作为肺细胞损伤的标志;BALF 中白细胞数和总蛋白含量的增加反映肺泡-毛细血管膜屏障的通透性增高。本研究结果表明,新生大鼠持续吸入高浓度氧,BALF 中 LDH 活性、总蛋白含量及白细胞总数明显增加,表明高氧暴露引起

新生大鼠肺组织细胞损伤及肺泡-毛细血管膜屏障通透性的增高。

谷氨酸是哺乳动物中枢神经系统重要的兴奋性神经递质,主要存在神经细胞内[7]。近年来研究发现,在外周非神经组织细胞中也存在谷氨酸受体[8],谷氨酸受体的过度激活可导致组织细胞不同程度的损伤[9]。申丽等[10]报道,腹腔注射谷氨酸可引起小鼠急性肺损伤,而谷氨酸 NMDA 受体拮抗剂 MK-801 可减轻谷氨酸所致的肺损伤,表明外源性高浓度的谷氨酸通过其 NMDA 受体可引起急性肺损伤。Said 等[11]报道,NMDA 受体阻断剂可以减轻百草枯(除草剂)和黄嘌呤氧化酶引起的氧化性肺损伤,表明谷氨酸 NMDA 受体的激活可引起氧化性急性肺损伤。本室前期的研究也发现,采用 MK-801 可有效减轻新生大鼠高氧性肺损伤[1],提示内源性谷氨酸通过其 NMDA 受体参与高氧性肺损伤的发生与发展。但在肺组织急性损伤时是否存在内源性谷氨酸的大量释放尚无相关报道。为此,本研究进一步观察了高氧暴露新生大鼠 BALF 中谷氨酸含量的变化。研究结果表明,持续高氧暴露 1 和 3 d,BALF 中谷氨酸含量明显升高,提示在高氧性肺损伤早期存在内源性谷氨酸的大量释放,但谷氨酸释放的细胞来源尚待进一步探讨。由于大鼠肺组织存在谷氨酸 NMDA 受体的表达[12],推测谷氨酸可能通过 NMDA 受体的内源性激活参与了高氧性肺损伤的发生与发展。这为 NMDA 受体参与高氧性肺损伤提供了依据,也为临床防治高氧性急性肺损伤提供新的思路。高氧暴露 7 d BALF 中谷氨酸含量低于正常对照组,其原因可能与高氧暴露早期肺内谷氨酸大量耗竭有关,其确切机制有待进一步阐明。

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Changes of endogenous glutamate release in lungs of newborn rats with hyperoxia-induced lung injury

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Abstract: **AIM** To demonstrate the role of intrinsic glutamate (Glu) in hyperoxia-induced lung injury. **METHODS** SD newborn rats, in 12 h after birth, were randomly divided into 2 groups: air control and hyperoxia (95% oxygen) groups. After 1, 3 and 7 d of continuous exposure to high concentration oxygen, the lungs of 5 rats in each group were removed. Then the wet-to-dry weight ratio (W/D) was measured and histopathological changes were observed with HE staining. The bronchoalveolar lavage fluid (BALF) of other rats in each groups was prepared and the leucocyte numbers, total protein (TP) concentrations and lactate dehydrogenase (LDH) activity in BALF was determined with hemocytometer, Lowry method and automatic biochemical analyzer, respectively. In addition, the level of Glu in BALF was determined by using high performance liquid chromatograph. **RESULTS** There was no difference in W/D between the air control and hyperoxia groups 1 d after hyperoxia exposure. After 3 and 7 d, W/D in hyperoxia groups were much higher than that of the air control group. HE staining showed that a few inflammatory cells appeared in some alveolar space in the hyperoxia group on 3 d. On 7 d, leukopedesis and red blood cells increased in the alveolar space, and there were fewer alveolai compared with control group. On 1 d, LDH activity in hyperoxia group was significantly higher than that in control group. The TP contents and leucocyte count had no obvious changes between the 2 groups. On 3 and 7 d, LDH activity, TP content and leucocyte count in hyperoxia groups were much higher than that of control group. In addition, the glutamate level in BALF on 1 and 3 d of hyperoxia exposure was significantly higher than that in control group. **CONCLUSION** Hyperoxia can induce acute lung injury and intrinsic Glu release in lungs of newborn rats, which show that Glu may play an important role in hyperoxia-induced lung injury.

Key words: hyperoxia; acute lung injury; glutamates

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