·Original Article in English·

Lung Injury of Preterm Rats Induced by Prolonged Exposure to High Oxygen Concentration of 85%

Li-Ling QIAN, Li-Wen CHANG, Qian-Shen ZHANG, Zhi-Hui RONG

Department of Pediatrics, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, *China*

Abstract: Objective To study the deleterious effect of prolonged hyperoxia exposure on preterm rat lungs. **Methods** On the 2nd postnatal day, preterm SD rats were randomly assigned to the air group (I) and hyperoxia group (II, exposed to 85% O_2). After 3, 7 and 14 days of exposure, the contents of total protein (TP), hydroxyproline (HYP) and malondialdehyde (MDA), total cell counts and differentiation in bronchoalveolar lavage fluid (BALF), ratio of lung wet weight/dry weight (W/D), and lung collagen content were examined. After 3, 7, 14 and 21 days of exposure, lung histopathology and radial alveolar counts (RAC) were performed. **Results** On day 3 of hyperoxia exposure, only the MDA content increased in Group II ($P<0.05$). On day 7 and 14, TP, HYP, total cell counts, the percentage of neutrophils in BALF and lung W/D also significantly increased ($P<0.05$ or 0.01). The differences of lung collagen contents between the two groups were not significant ($P>0.05$). Hyperoxia exposure resulted in subacute alveolitis and inhibition of lung development on day 7, 14 and 21. RAC was similar between the two groups on day 3 (4.9 ± 0.7 vs 5.0 ± 0.8), but different on day 7 (5.9 ± 0.9 vs 7.1 ± 0.9; $P<0.05$. On day 14 and 21, RAC decreased more obviously in Group II compared with that in Group I (7.0 ± 0.8 vs 9.9 ± 0.6, 7.3 ± 0.9 vs 10.5 ± 0.8; $P<0.01$). **Conclusions** Prolonged exposure to 85% O_2 may result in subacute inflammatory lung injury and inhibition of lung development in preterm rats. **[Chin J Contemp Pediatr, 2003, 5(2): 95-99]**

Key words: Hyperoxia; Lung injury; Preterm; Rat

85% 高浓度氧长期暴露诱发早产大鼠肺损伤

钱莉玲,常立文,张谦慎,容志惠 华中科技大学同济医学院附属同济医院儿科,湖北 武汉 430030

[摘 要] 目的 探讨长期高浓度氧(85%)暴露对早产新生大鼠肺组织的损伤作用。**方法** 早产SD大鼠生后第2天被随机分为Ⅰ空气组、Ⅱ高氧组(置85% O_2 中)。分别于暴露3,7,14 d后,检测支气管肺泡灌洗液(BALF)中总蛋白(TP)、丙二醛(MDA)、羟脯氨酸(HYP)含量和细胞总数及分类,肺组织湿重/干重(W/D),肺组织胶原含量;于暴露3,7,14,21 d后,行肺组织病理学检查和辐射状肺泡计数(RAC)。**结果** 3 d时Ⅱ组仅MDA含量增加($P<0.05$);7,14 d时,Ⅱ组BALF中MDA,TP,HYP含量、细胞总数、细胞分类中性粒细胞所占比例及肺W/D均明显增加($P<0.05$ 或<0.01)。两组肺胶原含量差异无显著性($P>0.05$)。除3 d外,Ⅱ组肺组织病理学检查可见不同程度的肺泡炎改变和肺发育滞后。7 d时Ⅱ组RAC值较Ⅰ组明显减少[(5.9 ± 0.9) vs (7.1 ± 0.9)]($P<0.05$);14,21 d时RAC值Ⅱ组较Ⅰ组[(7.0 ± 0.8) vs (9.9 ± 0.6);(7.3 ± 0.9) vs (10.5 ± 0.8)]减少更明显($P<0.01$)。**结论** 85% O_2 长期暴露,可引起早产新生大鼠亚急性炎症性肺损伤和肺发育受抑。

[中国当代儿科杂志,2003, 5(2): 95-99]

[关 键 词] 高浓度氧;肺损伤;早产;大鼠

[中图分类号] R-332 **[文献标识码]** A **[文章编号]** 1008-8830(2003)02-0095-05

Bronchopulmonary dysplasia (BPD) is a chronic lung disease that causes significant morbidity and

[Received] June 18, 2002; [Revised] October 12, 2002
[Foundation Item] Supported by the grant from Committe of Science and Technology of Hubei Province (No. 2000ZP1601)
[Introduction to the First Author] Li-Ling QIAN(1975-), Female, Master, Resident Doctor.
[Correspondence Author] Li-Ling QIAN, Department of Pediatrics, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China (Email: JSamicat@hotmail.com.)

mortality in infants. Despite recent improved neonatal care, including surfactant therapy and new ventilation modalities, the incidence of BPD is still prevalent. The precise etiology of the disease remains unclear. Prolonged exposure to hyperoxia in the newborn is believed to be the major contributing factor of the development of BPD[1]. In most instances, the animal models of hyperoxic lung injury used for studying BPD are established by short-duration exposure to oxygen (the concentration >90%) in adult or term newborn animals. However, the patients who require long-term supplemental oxygen are mostly premature infants. Therefore, we established a preterm rat model with hyperoxic lung injury induced by prolonged exposure to 85% O_2, which was more practicable in clinical practice.

Materials and methods

Animals and grouping

Adult Sprague-Dawley rats (weighing 200 - 220 g, 100 females and 34 males) were provided by the Experimental Animal Center of Tongji Medical College, and kept in the laboratory for mating. Fetal rats were delivered prematurely at gestational day 21 (term = 22 days) by cesarean section and kept by other dams for lactation. On the 2nd postnatal day, the preterm rats were randomly assigned to the air group (I) and hyperoxia group (II). The rats in Group II were kept in a chamber with an 85% O_2 concentration, a temperature of 25 - 26℃, a humidity of 60% - 70% and a CO_2 concentration of <0.5%. The rats in Group I were kept in room air.

Analysis of brochonalveolar lavage fluid (BALF)

After 3, 7 and 14 days of exposure, six rats from each group were sacrificed by an overdose of anaesthesia. The trachea was intubated. Following endotrachael intubation, the lungs were lavaged with sterile saline (about 0.1 ml/g) at 4℃. This procedure was repeated twice. All recovered fluid was collected. 100 μl of BALF was used to determine total cell counts with a hemocytometer counting chamber. Then the BALF was centrifuged for 10 min at 2500 rpm, and its cell debris was used for differential analysis by Wright-Giemsa staining. The supernatant fluid was stored at -20℃ and reused for measurement of total protein (TP), hydroxyproline (HYP) and malondialdehyde (MDA) (the assay kits were purchased from Jiancheng Bio Institute, Nanjing).

Measurement of lung wet weight/dry weight (W/D)

After 3, 7 and 14 days of exposure, another six rats of each group were killed. The right lung was then removed from the chest cavity, slightly blotted, and the wet weights were determined. Then the lung was dried in the oven at 60℃ and was weighed daily. The tissue was considered dry when the weight was constant for 2 consecutive days and the dry weight was recorded. Lung W/D was calculated.

Lung collagen measurement

The left lung was excised, slightly wiped and weighed. The lung tissue was crushed and lysed in 6 mol/L HCL at 110℃ for 24 h. The extraction was evaporated at 70℃, and then the residue was suspended in 0.5 ml distilled water. After purification, the content of hydroxyproline in the filtrate was measured by a spectrophotometer and the collagen content was determined after being multiplied by 7.46. The result was presented as mg/g lung tissue.

Lung histology and radial alveolar counts (RAC)

After 3, 7, 14 and 21 days of exposure, another six unlavaged rats of each group were killed. The lungs were perfused in situ via a tracheal cannula with freshly prepared 4% paraformaldehyde. The left bronchus was ligated, and then the left lung was excised, immersed in paraformaldehyde and embedded in paraffin wax within 24 h. 5 μm sections were stained with hematoxylin and eosin. Lung histology was examined under a light microscope, and radial alveolar counts (RAC) were performed by determining the number of septae that intersected a perpendicular line drawn from the center of a respiratory bronchiole to the distal acinus (connective tissue septum or pleura) under × 100 magnification[2]. At least 5 fields were scrutinized on each lung slide.

Statistical analysis

Data were presented as means and standard deviation (SD). The survival rate between groups was examined by χ^2 test. The differences of other data between the groups were assessed by Student t test.

Results

Animal survival rate

On the 3rd, 5th, 7th day of exposure, the survival rates in Group II (91.7%, 86.1%, 77.8%) were close to those in Group I (90.6%, 84.4%, 81.2%, respectively). On the 10th day of exposure to hyperoxia, the survival rate in Group II was lower than that in Group I (66.7% vs 78.1%; $P < 0.05$). On day 14 and 21 of O_2 exposure, the percentages of survivors in Group II dramatically decreased to 36.1% and 11.1%, significantly lower than those in Group I (78.1%, 78.1%; $P < 0.01$).

General conditions and body weights

The preterm rats in Group I had bright and smooth furs and plentiful subcutaneous fat and were active, while the rats in Group II had dry and dark furs and were emaciated, sluggish and inactive. About 14 days of hyperoxia exposure, the neonatal rats in Group II were obviously short of breath after being kept out of the oxygen chamber. All preterm rats continued to gain weight throughout the exposure period. However, the body weight and weight gain in Group II were significantly lower than those in Group I ($P < 0.01$). Moreover, with the passing time during hyperoxia, the weight differences between the two groups became more significant (See Table 1).

Table 1 Changes of body weight in each group (n=6, $\bar{x} \pm s$, g)

Group	0 d	3 d	7 d	14 d	21 d
I	3.76±0.29	7.07±1.19	11.28±0.94	21.85±1.65	40.73±1.77
II	3.81±0.32	5.19±0.82[a]	8.78±1.04[a]	17.77±1.14[a]	30.70±1.45[a]

Note: a vs Group I $P < 0.01$

TP, MDA, HYP, total cell counts and differentials in BALF

On day 3, only MDA increased in Group II compared with that in Group I ($P < 0.05$). On day 7 and 14, TP, HYP and total cell counts were also significantly increased ($P < 0.05$ or < 0.01). A difference was found in cell differentials between the two groups ($P < 0.01$ or 0.05) (See Table 2).

Table 2 TP, MDA, HYP, total cell counts and differentials in BALF and the lung W/D (n=6, $\bar{x} \pm s$)

Day	Group	HYP (μg/ml)	MDA (nmol/ml)	Cell count ($\times 10^5$/ml)	Differential (%)			W/D
					M	N	L	
3 d	I	0.50±0.12	0.31±0.18	3.0±0.4	96.4±3.0	2.2±0.5	1.4±0.4	4.98±0.14
	II	0.73±0.08	1.31±0.61[a]	3.3±0.6	95.5±2.8	3.2±0.8	1.3±0.6	5.08±0.13
7 d	I	0.46±0.05	0.31±0.20	2.6±0.4	96.1±3.0	2.1±0.5	1.8±0.4	4.96±0.11
	II	1.00±0.26[b]	1.67±0.66[b]	6.3±1.0[a]	51.3±3.2[b]	46.8±5.0[b]	1.9±0.5	5.21±0.11[a]
14 d	I	0.53±0.13	0.27±0.22	2.7±0.7	96.0±2.6	1.8±0.4	2.2±0.6	4.91±0.18
	II	1.83±0.53[b]	2.33±0.60[b]	14.6±2.0[b]	74.1±5.0[b]	23.1±8.0[b]	2.8±0.4	5.67±0.22[b]

Note: a vs Group I $P < 0.05$; b vs Group I $P < 0.01$

Lung W/D

As shown in Table 2, we did not find any differences in lung W/D between the two groups on day 3 ($P > 0.05$). On day 7 and 14, the ratios were significantly higher in Group II than Group I ($P < 0.05$ or 0.01).

Lung collagen

On day 3, 7 and 14, the lung collagen contents (mg/g. tissue) were 9.41 ± 0.23, 9.54 ± 0.37, 9.33 ± 0.34 respectively in Group I, and 9.39 ± 0.31, 9.44 ± 0.41, 9.53 ± 0.46 respectively in Group II. The differences between the two groups were not statistically significant ($P > 0.05$).

Lung histopathologic change and RAC

On day 3, no pathological change was observed in Group II. On day 7 and 14, red blood cells, monocytes, macrophages and edema fluid were seen infiltrating into the alveolar space and interstitium with thickened alveolar septa. On day 21, inflammatory

cell infiltration aggrevated markedly; and epithelial and fibroblast hyperplasia were seen. It also showed arrest of alveolar septation characterized as enlarged alveoli and lacking of homogenous small and middle-sized alveoli (See Figure 1). RAC was measured for quantifying the alveolar number within a terminal respiratory unit. RAC was similar between the two groups on day 3 (4.9 ± 0.7 vs 5.0 ± 0.8), but it was different on day 7 (5.9 ± 0.9 vs 7.1 ± 0.9; $P < 0.05$). On day 14 and 21, RAC decreased more obviously in Group II compared with that in Group I (7.0 ± 0.8 vs 9.9 ± 0.6, 7.3 ± 0.9 vs 10.5 ± 0.8), $P < 0.01$.

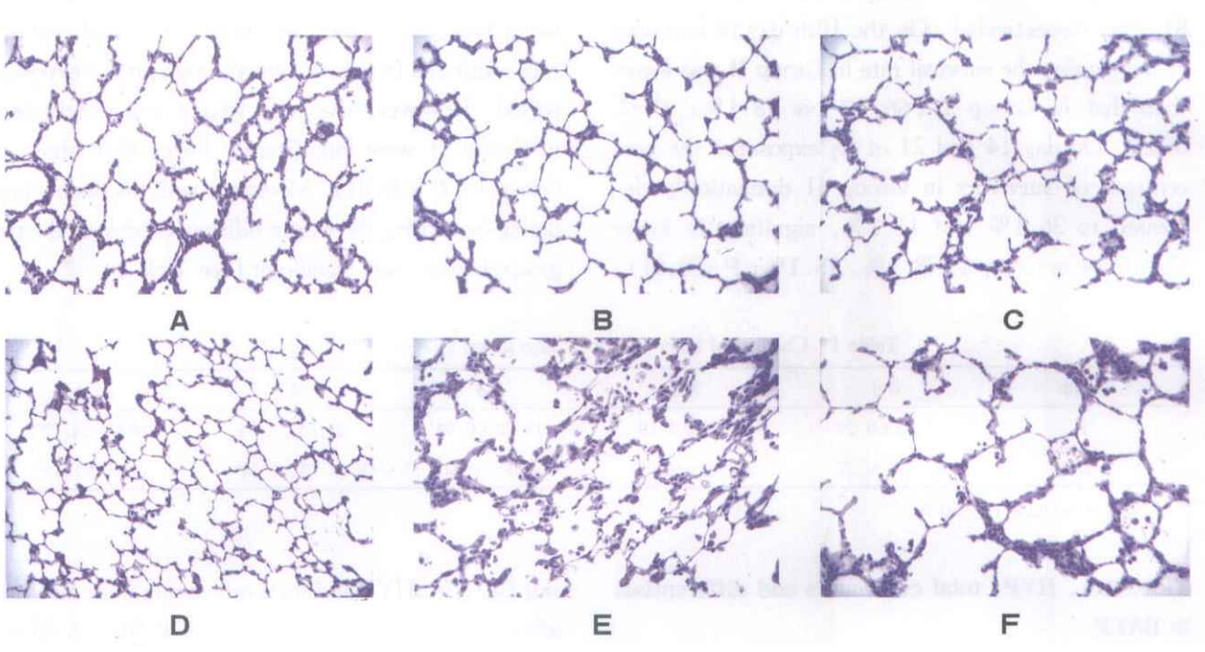

Figure 1 Histopathologic changes of lungs of preterm rats (HE×200)

A Hyperoxia group on day 3 (no obvious pathologic changes were found)

B Air group on day 7 (crests obviously increased and alveoli were forming)

C Hyperoxia group on day 7 (alveolar septa were slightly widened)

D Air group on day 21 (well developed alveoli were present)

E Hyperoxia group on day 21 (inflammatory cells markedly were infiltrated and epithelial cells proliferated increased)

F Hyperoxia group on day 21 (alveolar numbers decreased, alveolar structure were simplified, septa were widened and alveolar walls were thickened)

Discussion

Our results clearly showed that exposure to 85% O_2 in preterm rats did not result in significant difference in the survival rate compared with that of the air group during 7 days of exposure. We demonstrated a higher neonatal survival rate in hyperoxia compared with that reported in adult rats[3]. However, mortality was higher in the hyperoxia group than that in the air group after 7 – 10 days of exposure and the survival rate markedly decreased on the 14th day of O_2 exposure. Body weight gain was obviously lower in the hyperoxia group than that in the air group, suggesting that hyperoxia exposure resulted in retarded growth of the neonatal rats.

In our experiment, on day 3, MDA in BALF was already higher in the hyperoxia group than that in the air group. On day 7 and 14, HYP also increased significantly. These results revealed that exposure to 85% O_2 initially caused alveolar epithelial cell and vascular endothelial cell injury and indicated that MDA was a sensitive marker of early injury. With the passing time of hyperoxia exposure, the disruption of basement membranes occurred, lung permeability increased, and subsequently edema fluid and

inflammatory cells were infiltrated into the alveolar space and interstitium which resulted in the increase in total protein and total cell counts in BALF and lung W/D. The results demonstrated that exposure to 85% O_2 induced a subacute inflammatory response in the lung and suggested that the damage of the alveolocapillary membrane barrier contributed to the pathogenesis of lung injury. Our findings also showed that the percentage of neutrophils increased significantly after 7 days of O_2 exposure, but mononuclear-macrophage cells were still in abundance during the whole exposure period. Coalson et al[4]. have also reported that premature baboons demonstrated less injury and diminished neutrophils in the alveolar exudates compared with adult baboons after hyperoxia exposure. These results indicated that the pulmonary neutrophil response of neonates and adults differed. The probable mechanism is that the bone marrow of neonates is easily depleted, and additional neutrophils cannot be released into the circulation to reach the lung because the neutrophil storage pools are lower in the neonate.

It has been reported[5-6] that the exposure of adult animals to hyperoxia for 3 - 5 days could cause severe pathological changes characterized by pulmonary edema, hemorrhage, epithelial cellular necrosis and numerous extravasated inflammatory cells. Hyperoxia caused interstitial hyperplasia and fibrotic changes around the 14th day of exposure. Our pathological study showed that slight lung inflammatory response did not appear in preterm rats until being exposed to hyperoxia for 7 - 10 days. On day 14 and 21 of hyperoxia exposure, although cellular and collagen-like material hyperplasia, and an enlarged alveolar wall and lung septa were observed, no obvious fibrotic changes were found and pathological changes exhibited a significant alveolar septal arrest characterized by the reduction in the number of small-diameter alveoli and alveolar simplification or sacculation. In addition, no difference in lung collagen between the two groups also suggested no obvious collagen accumulation or fibrotic changes occurred. These results indicated that prolonged hyperoxia exposure induced neonatal lung injury characterized by subacute alveolitis, and inhibition of lung development, which is a special consequence of hyperoxic lung injury in neonatal animals. Furthermore, inflammatory response induced by hyperoxia exposure may be the critical factor, which contributes to the pathogenesis of inhibition of lung development in the newborn. Therefore, further elucidation of the mechanism of inflammatory response in related cells may provide a new approach in looking for therapeutic strategies for BPD.

Recently many investigators suggest that local emphysema, atelectasis and lung fibrosis are not the essential pathologic changes of BPD, and the arrest of lung development is just the primary histopathological characteristic[7-8]. The pathologic changes of our model were similar to the above-mentioned characteristics, which indicated that our present study may provide an ideal animal model for further exploring BPD.

[References]

[1] Saugstad OD. Bronchopulmonary dysplasia and oxidative stress: are we closer to an understanding of the pathogenesis of BPD? [J]. Acta Paediatr, 1997, 86(12): 1277 - 1282.

[2] Jakkula M, Le Cras TD, Gebb S, et al. Inhibition of angiogenesis decreases alveolarization in the developing rat lung [J]. Am J Physiol Lung Cell Mol Physiol, 2000, 279(3): L600 - 607.

[3] Yam J, Frank L, Roberts RJ. Oxygen toxicity: comparison of lung biochemical responses in neonatal and adult rats [J]. Pediatr Res, 1978, 12(2): 115 - 119.

[4] Coalson JJ, Kuehl TJ, Prihoda TJ, et al. Diffuse alveolar damage in the evolution of bronchopulmonary dysplasia in the baboon [J]. Pediatr Res, 1988, 24(3): 357 - 366.

[5] Crapo JD, Peters-Golden M, Marsh-Salin J, et al. Pathologic changes in the lungs of oxygen-adapted rats: a morphometric analysis [J]. Lab Invest, 1978, 39(6): 640 - 653.

[6] Thet LA, Wrobel DJ, Crapo JD, et al. Morphologic aspects of the protection by endotoxin against acute and chronic oxygen-induced lung injury in adult rats [J]. Lab Invest, 1983, 48(4): 448 - 457.

[7] Jobe AJ. The new BPD: an arrest of lung development [J]. Pediatr Res, 1999, 46(6): 641 - 643.

[8] Jobe AH, Ikegami M. Prevention of bronchopulmonary dysplasia [J]. Curr Opin Pediatr, 2001, 13(2): 124 - 129.

(Edited by Yan YU)