

Effects of High Concentration Oxygen Treatment on Traumatic Pneumothorax in Adult Rabbits

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Abstract

We undertook this study to investigate the adequate oxygen concentration that can be applied safely to the treatment of pneumothorax. Complete unilateral pneumothorax was induced artificially in rabbits, which were subsequently treated with various inspired oxygen fractions (FIO₂; 21%, 60%, 80% or 100%). The pneumothorax resolution time was measured together with the levels of IL-1 β and IL-8 in broncho-alveolar lavage (BAL) and plasma samples. Furthermore, the lungs from these animals were examined for histopathological evidence of oxygen toxicity. The results showed that the resolution time was significantly faster in the pneumothorax rabbits when treated with higher FIO₂. Significantly higher levels of IL-1 β were detected in BAL samples collected from the pneumothorax-rabbits that had received FIO₂ at levels of either 80% or 100% ($P < 0.05$), but not in those with FIO₂ at the 60% level. However, there was no significant change in the level of IL-8 in the BAL when the pneumothorax-rabbits were treated with different FIO₂ levels. In addition, no evidence of oxygen toxicity was found when the lung tissues were examined. The data indicated that higher FIO₂ treatment can accelerate the resolution of pneumothorax, but caution should be exercised with regard to associated oxygen toxicity when the FIO₂ used is greater than 80%. We conclude that treatment with 60% FIO₂ is an appropriate concentration for oxygen therapy for the treatment of pneumothorax in this model.

Key Words: broncho-alveolar lavage, inflammatory cytokine, IL-1 β , IL-8, hyperoxia.

Introduction

Pneumothorax is a medical emergency that occurs in patients with acute lung injuries (6, 14, 15, 22). The therapeutic options currently available for the management of pneumothorax include observation, supplementary oxygen, simple aspiration, intercostals

tube drainage and video-assisted thoracoscopic surgery (15, 22). Although oxygen may increase pneumothorax recovery rates, this beneficial effect is limited by the presence of short-term disadvantages as the potential long-term toxicity of unnecessary oxygen therapy (21, 24). The beneficial effect of hyperoxia in humans with pneumothorax has not been

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extensively studied. Chadha and Cohn reported that administration of high concentrations of inspired oxygen was an effective method of enhancing the resolution rate of pneumothorax in a study involving eight patients, especially when the pneumothorax was smaller than 30% (6). The only prospective human clinical trial of hyperoxia therapy involved ten participants with spontaneous pneumothorax of varying volumes and they were treated intermittently with high-flow oxygen for between 9 to 38 h (19). The concentration of oxygen was not measured but was frequently observed to be around 33% at 8 l/min (5). However, there is still a lack of an appropriate clinical study which can provide useful information concerning the optimal conditions of an effective oxygen therapy for pneumothorax (19, 23).

Hyperoxia can cause acute lung injury within a few days involving the initiation of the inflammatory cytokine cascade, which results in an increased alveolar epithelial permeability, recruitment of inflammatory cells from the blood into the alveolar space and the eventual endothelial and epithelial cell injury/death (1, 2, 4, 7, 9, 17, 20, 25). IL-1 β and IL-8 have been implicated in hyperoxia-induced acute lung injury. Previous studies have shown that elevated levels of IL-1 β and IL-8 are detectable in the bronchoalveolar lavage (BAL) of animals after exposure to hyperoxia (up to 95% FIO₂) which may contribute to the influx of inflammatory cells to the lung tissues (4, 9, 17, 20).

The object of this study is to determine the adequate oxygen concentration and duration for the treatment of pneumothorax. The levels of IL-1 β and IL-8 in the BAL and plasma samples were also determined as early biomarkers of hyperoxia-induced acute lung toxicity.

Materials and Methods

Animals

This study was approved by the Institutional Animal Care and Use Committee of the National Yang-Ming University in compliance with the international standard of laboratory animal usage. Forty adult white New Zealand rabbits (12 week old, weighing about 2.5 kg per animal) were used in this study. All rabbits were housed individually in standard plastic housing cages with two holes connected to the oxygen tanks. The gas exposure system consisted of a single-pass system with the exhaust air vented to the outside through chamber vents. The concentration of inspired oxygen within the animal cage was controlled by an oxygen sensor that was connected to the oxygen tank and set to the appropriate concentration of oxygen. The pressure inside the cages remained atmospheric.

Induction of Pneumothorax

Forty rabbits were included in this study among which 28 had induced artificially pneumothorax. All animals were anesthetized by an intramuscular injection of ketamine (44 mg/kg) and xylocaine (5 mg/kg). In the pneumothorax group, the right lungs of the anesthetized rabbits were penetrated with a sterilized 19G needle to generate an artificial pneumothorax. Four rabbits died within 3 h after induced artificial pneumothorax. The remaining 36 rabbits were divided into four groups each of which was treated with a different FIO₂ (21%, 60%, 80% and 100%). There were six rabbits with pneumothorax and three rabbits without pneumothorax, used as the control, in each group. A chest roentgenogram (General Electric, Pittsburgh, PA, USA) of every rabbit was performed initially to confirm the complete collapse of the right lung and was subsequently observed twice a day (at 9 am and 4 pm) to monitor the re-expansion of the collapsed lung. The resolution time of the pneumothorax was defined as the time from artificially induced pneumothorax to the complete re-expansion of right lung, as shown on a roentgenogram.

Collection of Broncho-Alveolar Lavage (BAL) Fluid and Plasma

BAL and plasma samples were collected from the rabbits when their pneumothorax had been re-expanded completely, as confirmed by roentgenogram. BAL was performed by injecting 5 ml of normal saline at 37°C into the lung through a tracheal cannula and BAL fluid samples were subsequently collected. This process was repeated six times in each rabbit. The recovery rate for the BAL fluid was about 70%. Blood was also drawn from the rabbits immediately after BAL sampling. The recovered BAL fluid and blood samples were then centrifuged at 1,500 g at 4°C for 3 min, and the supernatants of BAL and plasma were then harvested, aliquoted and then frozen at -70°C until use for cytokine analysis.

IL-1 β and IL-8 Protein Assays

The levels of IL-1 β and IL-8 proteins in the BAL and plasma samples from the rabbits were determined using enzyme-linked immunosorbent assay (ELISA) kits obtained from USCNLIFE Co, LTD (Missouri, TX, USA) according to the manufacturer's protocols.

Histopathology Analysis

Immediately after collection of BAL, the rabbits were deeply anesthetized with pentobarbital sodium (100 mg/kg body weight i.p.) and euthanized. After

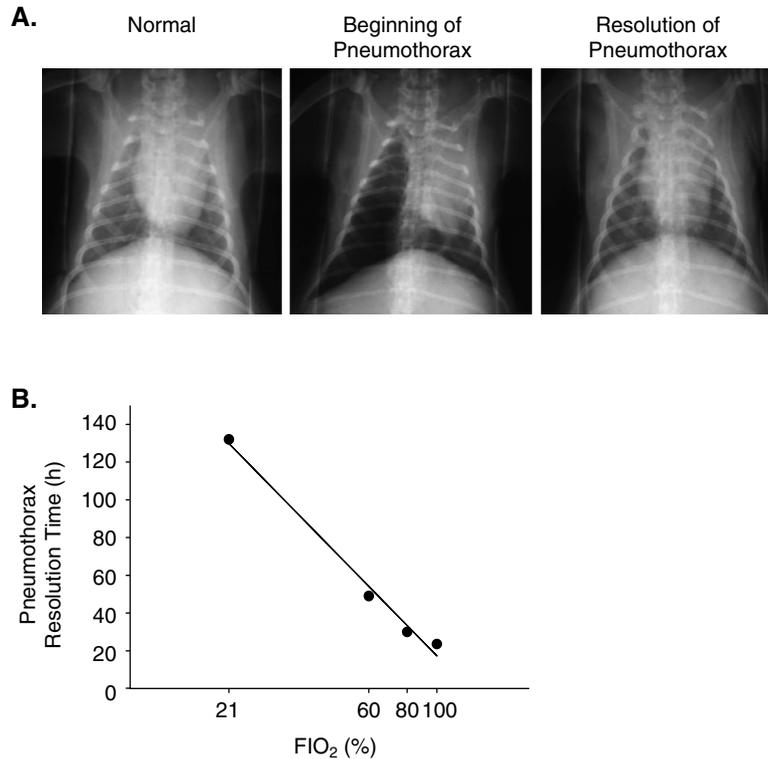


Fig. 1. Pneumothorax resolution time was significantly shortened by hyperoxic treatment. Rabbits were housed in an oxygen chamber at four designated FIO₂ (21%, 60%, 80% and 100%) after induction of a complete unilateral pneumothorax. (A) Chest roentgenograms were obtained twice a day until the pneumothorax was resolved. (B) There was an inverse relationship between inhaled oxygen concentration levels (FIO₂) and the resolution time of pneumothorax. (Correlation coefficient = -0.955 and $P = 0.045$; Pearson Product Moment Correlation).

ligation of the trachea, the lung was removed *en bloc*, immersion fixed in formalin for 16-20 h, washed and stored in 0.1 M cacodylic acid at 4°C. The samples were then dehydrated and embedded in paraffin. The lung samples from both pneumothorax and non-pneumothorax sides were sectioned at a thickness of 4-6 μm for the study. The sectioned samples were stained with hematoxylin and eosin stain and examined under a microscope. Masson's trichrome staining was also performed to evaluate the degree of collagen deposition and fibrosis.

Data Analysis

The data of resolution time were evaluated using Kruskal-Wallis ANOVA. The results of cytokines were analyzed by Mann-Whitney test. A P value of less than 0.05 was considered statistically significant. All data are presented as means \pm SEM.

Results

Higher Concentration of Oxygen Treatment Is Inversely Associated with a Reduction in the Resolution Time of Pneumothorax

Fig. 1 shows a representative roentgenogram of a pneumothorax as found in our study and shows that the collapsed lung has re-expanded completely after hyperoxic therapy. No evidence of pleural effusion was found in any of the pneumothorax rabbits when their lung had re-expanded completely. All pneumothorax and non-pneumothorax rabbits were alive during the experiment and observation period. When the resolution time after treatment was determined for the different concentrations of supplemental oxygen, the results were as follows: (1) room air (21% FIO₂): 132.0 \pm 11.2 h ($n = 6$); (2) 60% FIO₂: 49.0 \pm 2.4 h ($n = 6$); (3) 80% FIO₂: 30.0 \pm 0 h ($n = 6$) and (4) 100% FIO₂: 23.6 \pm 0.5 h ($n = 6$). The resolution time was significantly faster in the pneumothorax rabbits when they were treated with the higher concentrations of oxygen ($P < 0.001$; Kruskal-Wallis ANOVA test) and there was an inverse relationship between the inhaled oxygen concentration level and the pneumothorax resolution time (Pearson Product Moment Correlation; correlation coefficient = -0.955 and $P = 0.045$; Fig. 1B).

The Levels of IL-1 β and IL-8 Proteins in the BAL

In order to determine whether or not hyperoxia-

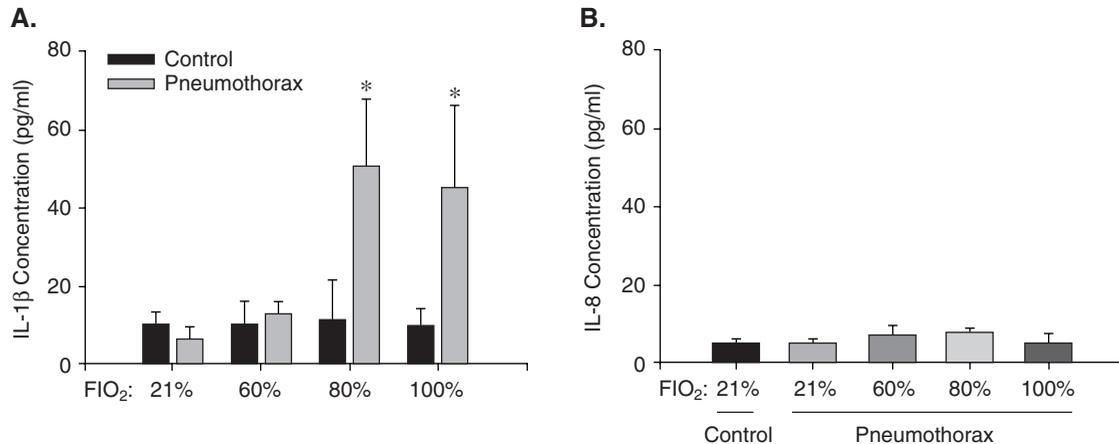


Fig. 2. Cytokine levels in the broncho-alveolar lavage. Broncho-alveolar lavage was collected from the pneumothorax rabbits (N = 6) when their collapsed lungs had re-expanded completely as confirmed by chest roentgenogram, and also in the control rabbits (N = 3). The levels of IL-1 β (A) and IL-8 (B) in BAL were analyzed by ELISA. (* $P < 0.05$; Mann-Whitney test).

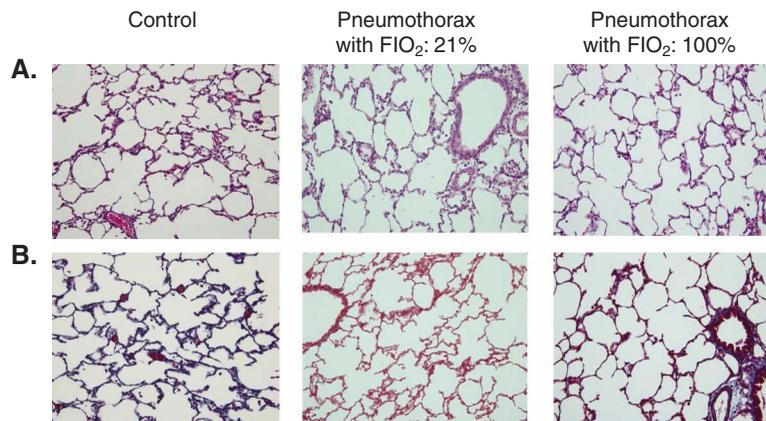


Fig. 3. Histopathology of the rabbit lung tissues. Rabbit lung tissues were sampled from the control rabbits without pneumothorax (left column) and from the pneumothorax rabbits that had received either FIO₂ 21% (center column) or higher FIO₂ (right column) treatment. The lung tissues were stained with hematoxylin and eosin stain (A; 100 \times) and Masson's trichrome (B; 100 \times).

induced lung toxicity had been developed, the levels of two cytokines in the BAL were determined. The BAL samples were collected from the control and the tested rabbits immediately after complete re-expansion of collapsed lungs. Our results showed that samples from rabbits treated with higher FIO₂, either at 80% or 100%, had significantly higher levels of IL1 β comparing to the control rabbits (without pneumothorax) who received the same FIO₂ ($P < 0.05$; Fig. 2A). However, when the groups receiving either FIO₂ 21% or FIO₂ 60% were examined, there was no significant difference between the pneumothorax group and the control group. We also determined the level of IL-8 in the BAL samples. However, there was no significant difference in the level of IL-8 in the BAL when the rabbits with pneumothorax were treated with FIO₂ (Fig. 2B) at all concentrations. The plasma levels of IL-1 β and IL-8 were not detectable among rabbits

receiving different concentrations of oxygen (data are shown).

Histopathology

Lung tissues from the rabbits in the various groups were also examined histologically under a microscope for evidence of oxygen toxicity. No significant increase in the number of inflammatory cells or in the amount of fibrosis was found in any of the lung tissue samples from either the pneumothorax or non-pneumothorax animal after exposure to the therapies of different concentrations of oxygen (Fig. 3).

Discussion

In the current study, we have demonstrated that a higher FIO₂ (60% to 100%) is able to significantly

shorten the resolution time of pneumothorax. Our results show that supplemental oxygen therapy in pneumothorax rabbits at FIO₂ levels of 60%, 80% or 100% is able to improve the resolution rates by 2.7-fold, 4.4-fold and 5.5-fold, respectively, comparing to those of rabbits receiving room air (FIO₂ 21%) only. These results are consistent with the findings reported by Zierold *et al.* who also found that supplemental FIO₂ (40% and 60%) accelerated the resolution rate by 1.5-fold and 2.8-fold, respectively (26). Other studies have also confirmed that supplementary oxygen facilitates the resolution of injury-induced pneumothorax (11, 16). Previous studies have indicated that inhalation of a high concentration of oxygen may reduce the total pressure of gases in pleural capillaries by reducing the partial pressure of nitrogen. This should increase the pressure gradient between the pleural capillaries and the pleural cavity, thereby increasing absorption of air from the pleural cavity (12, 18).

Although supplemental oxygen therapy may be able to accelerate the resolution of pneumothorax, its clinical use is limited by the possibility of hyperoxia-induced lung toxicity. Our results show that the level of IL-1 β is elevated in BAL collected from the pneumothorax groups treated with FIO₂ either at 80% concentration for 30 h or at 100% for 24 h. These kinetics are much earlier than those reported in a previous study which shows that IL-1 β mRNA expression has been enhanced by 7 fold but only after 4 days of hyperoxic exposure (FIO₂ >95%) in adult mice (20). The reason for the relatively early elevation of IL-1 β in BAL in our study is not clear. It seems unlikely that the elevation in IL-1 β level was caused by the short term exposure of hyperoxia in our study because the control rabbits without pneumothorax did not have such an elevation after being treated with FIO₂ for the same duration (Fig. 2A). Another factor that may be related to this event is the rapid re-expansion of the collapsed lungs, which could induce minor trauma due to rapid stretching of the collapsed alveolar walls or rapid re-perfusion of the alveolar vessels. IL-1 β is known to be up-regulated in the lung tissues upon re-expansion and ventilation after a short-period of lung collapse and this has been implicated in the development of re-expansion pleural effusion (13). However, there was no evidence of re-expansion pleural effusion in our study rabbits. Thus, it is difficult to extrapolate the consistent elevation of IL-1 β in the present study to this rare complication of pneumothorax. Furthermore, it is also unlikely that the elevation of IL-1 β is induced by the pneumothorax itself because De Smedt *et al.* have reported previously that no significant change can be detected in the level of IL-1 β in the BAL harvested from patients with spontaneous pneumothorax (10). In a previous study, it was demonstrated that the levels of IL-8 in BAL

were elevated after 6 to 10 days of exposure to hyperoxia, and that the elevated IL-8 levels in the BAL coincided with a neutrophil recruitment to the BAL (8). However, our results suggest that short-term hyperoxic treatment (FIO₂ 60% - 100%) for 24 to 49 h was not associated with an elevated level of IL-8 in BAL when it was measured after the pneumothorax had been resolved completely. This implies that a short term (less than 2 days) beneficial effect of hyperoxic treatment for pneumothorax is possible. In addition, hyperoxic treatment has been implicated to play a role in many other clinical conditions such as tissues hypoxia and infections in order to accelerate a tissue repair process (3). Despite this beneficial effect, high FiO₂ oxygen therapy should be used with caution in patients with secondary pneumothorax caused by cystic fibrosis, advanced bronchiectasis or chronic obstructive pulmonary disease because these patients are commonly associated with hypercapnia (6, 15, 22).

Rapid removal of pleural air either by tube thoracostomy/pigtail drainage or surgical intervention has been the treatments of choice for total lung collapse in routine practice. The present results have demonstrated the efficacy of high FIO₂ to accelerate the resolution of pneumothorax. We conclude that short term 60% FIO₂ is an appropriate concentration for oxygen therapy, which is easily available and can be rapidly applied to patients while waiting for surgical intervention. Our data may lend support to future clinical trials for assessing the beneficial effect of supplemental hyperoxic treatment on pneumothorax resolution.

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