行政院國家科學委員會專題研究計畫　成果報告

吸入性一氧化氮對高濃度氧氣引發小鼠呼吸道高反應性及急性肺損傷之影響

計畫類別： 個別型計畫
計畫編號： NSC91-2314-B-002-281-
執行期間： 91 年 08 月 01 日至 92 年 07 月 31 日
執行單位： 國立臺灣大學醫學院內科

計畫主持人： 郭炳宏

報告類型： 精簡報告

處理方式： 本計畫可公開查詢

中　華　民　國 92 年 10 月 13 日

計畫名稱: 吸入性一氧化氮對高濃度氧氣引發呼吸道高反應性及急性肺損傷之影響

Effects of inhaled nitric oxide on the survival and apoptosis of hyperoxide-induced lung injury in mice

計畫編號：91-2314-B-002-281-
執行期限：91 年 8 月 1 日至 92 年 7 月 31 日
主持人：郭炳宏　國立台灣大學醫學院內科

一、 摘要

關鍵詞：高濃度氧氣, 呼吸道高反應性. 吸入性一氧化氮, 急性肺傷害, 細胞凋亡

]

過去的研究顯示高濃度氧氣可引起呼吸道反應性增高 (airway hyperresponsiveness)**，肺部順應性**(compliance)**下降，嗜中性白血球浸潤，肺水腫，透明膜**(hyaline membrane)**形成而導致呼吸衰竭。許多小型哺乳類動物在高濃度（**>95%**）氧氣中數天內就會死亡。然而，經過近** 30 **年的研究，高濃度氧氣形成急性肺傷害的詳細致病機轉仍未清楚。吸入性一氧化氮**(NO)**常被用來在急性肺傷害或肺動脈高壓的病患以提高血氧分壓，也常與高濃度氧氣合併使用。究竟吸入性** NO **對高濃度氧氣傷害有無益處目前仍是個謎。我們發現吸入性** NO **可顯著延長小鼠在＞**95**%高濃度氧氣中之存活率。而且** 20 **及** 60 ppm **的** NO **可降低呼吸道高反應性與肺泡灌洗液中嗜中性白血球之數目。此外，**BAL F **中蛋白質，**8isoprostane **及過氧化氫（**H_2O_2**）的濃度也隨** NO **之劑量而減少。肺部切片中肺發炎之程度與** TUNEL **染色呈陽性的細胞數也有同樣趨勢之變化。顯示吸入性** NO **有減少高濃度氧氣引發肺部細胞凋亡及肺發炎之作用。希望此一動物實驗結果可對吸入性** NO **在氧氣毒性的療效及機轉釐清方面有所幫助。**

二、 緣由與目的

Hyperoxia (>95% O_2) generates reactive oxygen species, causing pulmonary pathology and dysfunction, and leading to death in most mammalian species. Manifestations of pulmonary oxygen toxicities include enhanced airway hyperresponsiveness (AHR), decreased compliance, pulmonary edema, increased capillary permeability, and hyaline membrane formation. After nearly 30 years of research, however, the precise mechanisms of hyperoxia-induced toxicity have not been completely clarified. Inhaled nitric oxide (NO) is often used in acute lung injury because it can lower O_2 requirements due to its action

as a selective and potent pulmonary vasodilator. There are presently only a limited number of studies on the influence of inhaled NO on hyperoxia-induced lung injury and the results were controversial. Gutierrez et al. (1996) found increased survival in rats at 144 h with the addition of 7.8 ± 0.2 ppm NO to >95% O_2, whereas Garat et al. (1997) found no effect of either 10 ppm NO or 100 ppm NO on survival. A recent study showed that short-term (6 h) exposure to NO/O_2 delayed the onset of respiratory distress but did not exacerbate nor attenuated pulmonary dysfunction. From a clinical perspective, we think it is important to address the following questions. (1) Does inhaled NO improve the survival of hyperoxia-induced lung injury? (2) Are there differences between the effects of high and low doses of inhaled NO? (3) Through which mechanism(s) does inhaled NO affect the survival of animals exposed to hyperoxia?

The aims of this study were to investigate the effects of inhaled NO on the hyperoxia-induced lung injury. Effects on oxidative stress were assessed using the levels of H_2O_2 and 8-isoprostane.

三、 材料及方法:

Adult BALB/c mice (7-8 weeks of age) were exposed to 4 experimental conditions: (1) Normoxia (room air); (2) Hyperoxia (> 95% O_2); (3) Hyperoxia + iNO 20 ppm; and (4) Hyperoxia + iNO 60 ppm.

Animal Exposure and Handling: A 60 x 48 x 25-cm plastic exposure chamber with 12 h/12 h light-dark cycles. Humidified pure oxygen or air was delivered to the chamber at a flow rate of 2.5-3 liters/min. The oxygen concentration for all experiments ranged from 95 to 99%. NO was delivered to the chamber by a specialized NO delivery system (Taema opti-NO, Detendeur HBS-CL 3100, UK). NO and NO_2 levels were monitored using a chemoluminescent monitor.

Airway hyperresponsiveness (AHR) : AHR was assessed in 5 conscious mice in each group at 0, 24^{th} hr and 96^{th} hr by whole body plethysmography (Buxco). Bronchoalveolar lavage:. Liu's stain for differential cell analysis. Protein concentration : method of Bradford. H_2O_2 measurements: 100 µl of BAL supernatant was mixed with 100 µl of 420 µM 3'3'5'5'-tetramethylbenzidine in 0.42 M citrate buffer pH 3.8 and 10 µl of horseradish peroxidase (52.5 U/ml). The samples were incubated at room temperature for 20 min and the reaction stopped by the addition of 10 of 18 N sulfuric acid. The product was measured

spectrophotometrically (ELISA reader) at 450 nm for each assay. The level of 8-isoprostane was determined by an EIA-kit. (Caymen Co).

Assessment of Apoptosis: TUNEL staining (TdT-FragEL DNA Fragmentation Detection KIT, Oncogen). DNA Fragmentation :TACS apoptotic DNA laddering kit

Statistical Analyses; Kapln-Mier analysis and ANOVA.

四、結果與討論

We have created an animal model of hyperoxia-induced acute lung injury. All mice exposed to hyperoxia (condition 2) died within 4-6 days, with enhanced AHR, lung edema and neutrophil inflammation after 48 h. Both concentrations of iNO prolonged the survival of mice exposed to hyperoxia (both $p < 0.001$) (Figure A). There were significant reductions in the dry weight of lung tissues (Figure B). Inhaled NO also attenuated AHR and reduced neutrophil counts (Figures C-E) and concentrations of total protein (Figure F), 8-isoprostane and H_2O_2 (Figure G) in the BAL fluid in a dose-response fashion. scores of lung inflammation as well as the number of TUNEL-positive cells on the pathology slides (Figure H).

These data indicate that iNO at 20 and 60 ppm prolong survival and attenuate AHR, lung edema and lung inflammation in mice exposed to hyperoxia, probably through the protective effects on oxidative stress and apoptosis.

參考文獻 (References)

Hershenson, M, Aghili S, Punjabi N, et al. Hyperoxia-induced airway hyperresponsiveness and remodeling in immature rats. *Am J Physiol Lung Cell Mol Physiol* 262: L263-L269, 1992

Gaboury, J, Woodman R, Granger D, et al. Nitric oxide prevents leukocyte adherence: role of superoxide. *Am J Physiol Heart Circ Physiol* 265: H862-H867, 1993

Nelin, LD, Welty S, Morrisey J, et al. Nitric oxide increases the survival of rats with a high oxygen exposure. *Pediatr Res* 43: 727-732, 1998

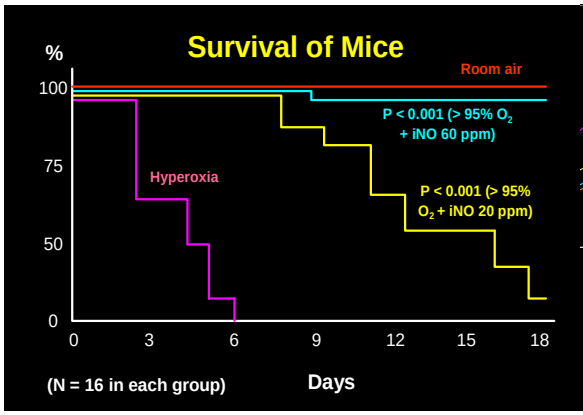


Figure (A)

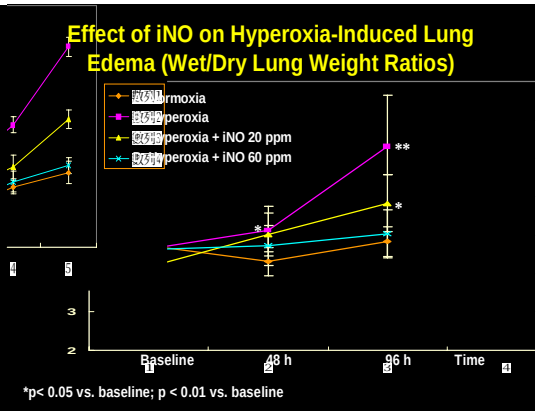


Figure (B)

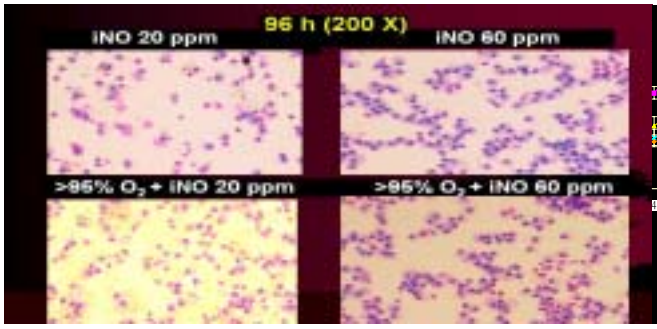


Figure (C)

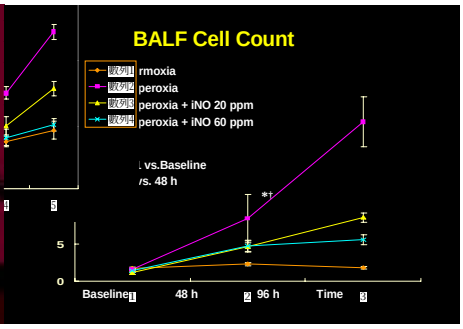


Figure (D)

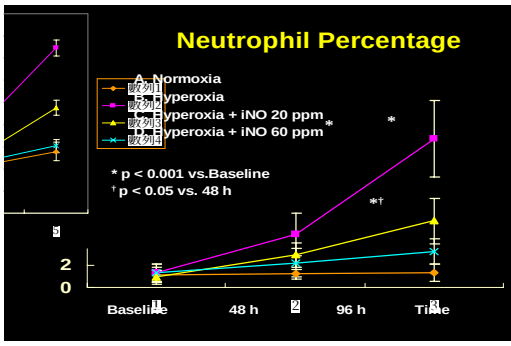


Figure (E)

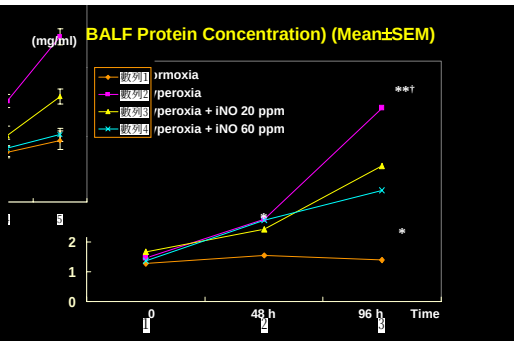
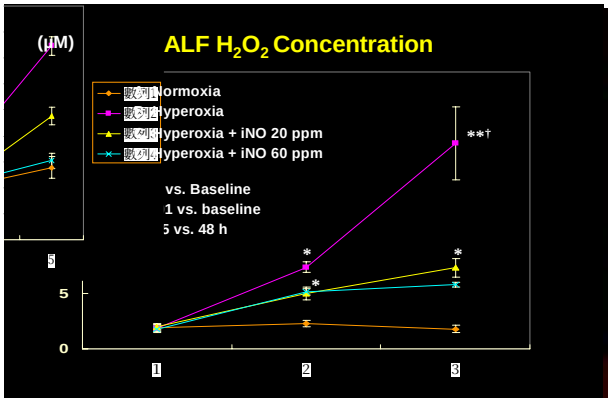


Figure (F)



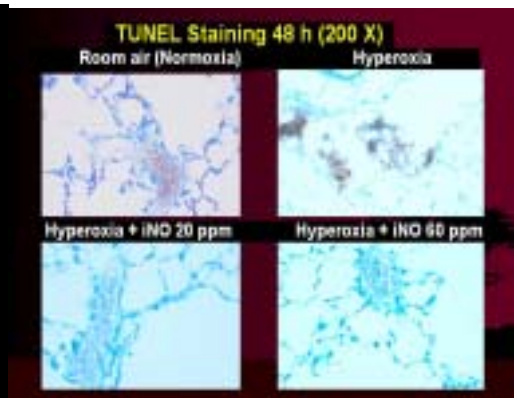


Figure (G)

Figure (H)