Chinese Journal of Physiology 52(3): 115-127, 2009
DOI: 10.4077/CJP.2009.AMH025

Enhancement Effects of Hypercapnia on the Acute Lung Injury Caused by Acid Aspiration

Demeral David Liu[1, 2, 3], Hen I Lin[4], Nan-Kuang Hsieh[5], and Hsing I Chen[6]

[1]*Department of Dentistry*
[2]*Neuro-Medical Scientific Center, Tzu Chi University and Hospital, Hualien*
[3]*Department of Dentistry and Center for Neuropsychiatry, China Medical University Hospital, Taichung*
[4]*Division of Chest Medicine, Cardinal Tien Hospital and Fu-Jen Catholic University, Taipei*
[5]*Department of Family Medicine, Tao-Yuan General Hospital, Department of Health, Executive Yuan, Taoyuan*
and
[6]*Institute of Integrative Physiology and Clinical Sciences, Tzu Chi University, Hualien, Taiwan*

Abstract

Acid aspiration or intrapulmonary instillation of gastric particles causes lung inflammation leading to acute lung injury (ALI). Hypercapnia exerts different effects on ALI caused by various insults. The effects of hypercapnia on lung inflammation and injury due to acid aspiration are yet to be determined. The present study was designed to investigate the involvement of inducible nitric oxide synthase (iNOS) and other mediators in acid-aspiration-induced ALI. We also sought to evaluate the effects of hypercapnia on the lung and associated changes induced by acid aspiration. We used Spague-Dawley rats anesthetized with intraperitioneal pentobarbital (40 mg/kg). Gastric acid particles were prepared from the stomach contents of rats at necropsy. The rats were randomly assigned to receive intratracheal instillation of physiological saline solution (PSS) at pH 7.24 (Control group), PSS at pH 1.25 (Low pH, LPH group), gastric particles (GP group), and GP with low pH PSS (GPLPH group). There were 10 rats in each group. The animals were observed for 6 hrs. To evaluate the effects of hypercapnia, we carried out two series of experiments: one under normocapnia and the other under hypercapnia with alteration of CO_2 fraction in inspired air. Arterial pressure (AP) was monitored from the femoral arterial catheter. Heart rate was obtained from AP traicing. We determined the blood gases and acid-base status. Lung weight to body weight (LW/BW) ratio, LW gain (LWG), protein concentration in bronchoalveolar lavage (PCBAL) and leakage of Evans blue dye tracer were measured. Plasma nitrate/nitrite, methyl guanidine (MG), myeloperoxidase (MPO), phospholipase A_2 (PLA_2), pro-inflammatory cytokines were assessed. Histopathological examination of the lung tissue was performed. We employed reverse-transcriptase polymerase chain reaction to detect the expression of iNOS mRNA. GP and GPLPH caused hypotension, decreases in PaO_2, pH and SaO_2, and an increase in $PaCO_2$. The insults also elevated LW/BW, LWG, PCBAL and dye leakage, plasma nitrate/nitrite, MG, MPO, PLA_2, tumor necrosis factor$_\alpha$, interleukin-β and interleukin-6. The lung pathology was characterized by alveolar edema and hemorrhage with inflammatory cells infiltration. Assessment of lung injury score revealed that GP and GPLPH caused ALI. Furthermore, hypercapnia significantly enhanced ALI and associated changes following LPH, GP and GPLPH. Intratracheal instillation of GP in normal or low pH PSS causes ALI accompanied with biochemical changes. The release of nitric oxide *via* iNOS isoform is detrimental to the lung. Hypercapnia tended to enhance ALI and associated changes induced by gastric acid instillation.

Corresponding author: Hsing I Chen, MD, PhD, Institute of Integrative Physiology and Clinical Sciences, Tzu Chi University, No. 701, Section 3, Jhongyang Rd, Hualien 97004, Taiwan. Tel: +886-3-8560824, Fax: +886-3-8573053, E-mail: chenhi@mail.tcu.edu.tw
Received: April 28, 2008; Revised: October 15, 2008; Accepted: November 14, 2008.
 ISSN : 0304-4920. http://www.cps.org.tw

Key Words: acute lung injury, acid aspiration, nitric oxide, inducible nitric oxide synthase, pro-inflammatory cytokines, hypercapnia

Introduction

Gastric aspiration occurs frequently in surgical patients under anesthesia and other causes such as blunt thoracic trauma, impaired glottic competency, and pregnancy (33, 40). It is one of the major causes of acute respiratory distress syndrome (ARDS) (35, 50). Intratracheal instillation of hydrochloric acid (HCl) or gastric particles has been employed as experimental model of acute lung injury (ALI) (6, 15, 43).

Recruitment and activation of polymorphonuclear cells such as neutrophils and macrophages have been implicated in the pathogenesis of ALI caused by acid aspiration (5, 32). However, the ultimate mechanism of inflammatory responses to acid challenge in the lung remains unclarified.

The present study was designed to elucidate the involvement of inducible nitric oxide synthase (iNOS) and other mediators in the acid aspiration ALI. Nitric oxide (NO) production through the iNOS system has been shown to be detrimental to the lung tissue in ALI due to various causes (2, 8, 9, 14, 19, 42, 46). Jian and coworkers have found that ALI induced by acid aspiration was attenuated by nonspecific and specific iNOS inhibitors (20). They suggest that NO generation through iNOS isoform is involved in the acid aspiration ALI. However, they did not provide evidence to indicate the upregulation of iNOS mRNA. In the present investigation, we employed reverse-tanscriptase polymerase chain reaction to detect the expression of iNOS mRNA.

The second aim of the study was to investigate the effects of hypercapnia on ALI following acid aspiration. Deliberate induction of hypercapnic acidosis exerts protective effect on ALI following endotoxin hyperventilation or mesenteric ischemia-reperfusion (7, 26, 27). Whether hypercapnic acidosis affects acid-induced inflammatory changes in the lung is an issue that requires evaluation.

Materials and Methods

Animal Preparation

We used male Spague-Dawley (SD) rats, 12-14 wk-old, weighing 360-380 g. The animals were obtained from the National Animal Center and housed in the University Laboratory Animal Center with good environment control. The animal experiment was approved by the University Committee of Laboratory Animal Care and Use, and followed the guidelines of the National Animal Research Center. The room temperature was maintained at 21 ± 1°C under a 12/12 h light/dark regimen. Food and water were provided *ad libitum*.

The animals were anesthetized with sodium pentobarbital (40 mg/kg, intraperitoneal injection). A femoral artery was cannulated and connected to a pressure transducer (Gould Instruments, Cleveland, OH, USA) to record the arterial pressure (AP) and heart rate (HR) on a polygraph recorder (Power Lab AD Instruments, Mountain View, CA, USA). A femoral vein was catheterized for intravenous (i.v.) administration of test agents or fluid.

The animals were intubated with an endotracheal tube and artificially ventilated with a rodent respirator with room air. The tidal volume and respiratory rate were 2.5-3.2 ml and 60-70 breaths/min, respectively. End-expiratory pressure was kept at 2 cm H_2O.

Instillation of Gastric Acid Particles

We prepared gastric acid particles from the stomach contents of SD rats at necropsy in accordance with the procedures described previously (15, 25). Particles were washed three times in physiological saline solution (PSS, pH 7.28) by filtration through sterile gauze sponges to remove debris, autoclaved for 25 min (20 psi, 121°C), pelleted by centrifugation at 2,000 g for 2 min, and resuspended in PSS. The particle diameter averaged 10 μM. The gastric acid particles were instilled into the trachea.

Blood Gases and Acid-Base Status

Arterial blood samples (0.5 ml) were taken from the femoral arterial catheter for the determination of blood gases and acid-base status (pH, PaO_2, $PaCO_2$, and SaO_2,) with a pH and blood gas analyzer (ABL-30 blood gas analyzer, Radiometer, Copenhagen, Denmark).

Measurements of Acute Lung Injury
Lung Weight to Body Weight (LW/BW) Ratio and Lung Weight Gain (LWG)

Lung weight (LW) was obtained at the end of the experiment. However, the initial LW could not be obtained before the experiment. We employed the method described previously to estimate the initial LW (11). Both LW and BW were obtained from 30 rats sacrificed by decapitation. The LW was then

plotted against BW for a regression equation.

$$LW\ (g) = 0.0015 \times BW\ (g) + 0.034$$

LWG was calculated as:

$$LWG\ (g) = \text{final LW} - \text{initial LW}$$

Protein Concentration in Bronchoalveolar Lavage (PCBAL)

At the end of the experiment, PCBAL was measured. The lungs were lavaged twice with saline (2.5 ml per lavage). Lavage samples were centrifuged at 1,500 g at room temperature for 10 min. The PABAL was determined with a spectrophotometer by measuring the change in absorbance at 630 nm with the addition of bromocresol green (21).

Leakage of Evans Blue Dye

Evans blue (30 mg/kg) was injected intravenously 30 min before the end of experimentation. The lungs were harvested and homogenized. Evans blue was extracted in formamide, and quantified by measuring optical density at 620 nm (47).

Blood Sample Analysis

Blood sample (0.5 ml) was taken from the femoral vein catheter and centrifuged at 3,000 g for 10 min. The supernatant was used for the measurement of nitrate/nitrite with high-performance liquid chromatography (ENO-20, AD Instrument, Tokyo, Japan) (36). The formation of methyl guanidine (MG) is an index of hydroxyl radical (38). It was determined using a fluorescence spectrophotometer (Jasco 821-FP, Spectroscopic CO, Tokyo, Japan). The emission and excitation maximums were set at 500 nm and 390 nm, respectively. The assay was calibrated with authentic MG (Sigma M0377).

To measure the myeloperoxidase (MPO) activity, the samples were mixed with 2 ml of potassium phosphate buffer (50 mM, pH 6.02) containing 0.5% cetyltrimethylammonium bromide and were centrifuged at 2,500 g for 10 min at 4°C. The supernatant was diluted with dilution buffer, then mixed with an assay buffer composed of 0.00107% H_2O_2 in potassium phosphate buffer and o-dianisidine. The reaction mixture was incubated at room temperature. The change in absorbance at 450 nm over 1 min was detected spectrophotometrically using the absorbance of MPO standard (Elastine Products, Detroit, MC., USA). The procedures basically followed those by Gray *et al*. (18).

Plasma concentration of phospholipase A_2 (PLA_2) was measured on a spectrofluorimeter using a method described previously (23, 24). In brief, the standard incubation mixture contained 10 mM Tris/HCl buffer (pH 7.4) with 2 μM Ca^{2+} and 5 μM C_{12}-NBD-PC-{1-palmitoyl-2[12-[7-nitro-2-1,3-benzoxadiazol-4yl]amino]hexanyl]-sn-glycero-3-phosphocholine} as the substrate. The absorbance of the reaction mixture was measured with excitation and emission wavelengths at 475 and 535 nm, respectively.

Determination of Pro-Inflammatory Cytokines

Tumor necrosis factor$_\alpha$ (TNF_α), interleukin-1β (IL-1β), interleukin-6 (IL-6) were measured with antibody enzyme-linked immunosorbent assays (ELISAs) with a commercial antibody pair, recombinant standards, and a biotin-streptavidin-peroxidase detection system (Endogen, Rockford, IL, USA). All agents, samples, and working standards were prepared at room temperature according to the manufacturer's directions. The optical density was measured at 450/540 nm wavelengths by automated ELISA readers.

Histopathological Examination

In rats which died following acid particle instillation and at the end of 6 h treatment, the lungs were removed. The lungs were cut into small pieces and immersed in 10% formaldehyde for 24 h. The lung tissue was dehydrated with graded alcohol and then embedded in paraffin at 60°C. A series of microsections (5 μm) were stained with hematoxylin and eosin. For a quantitative evaluation of the lung pathology, we employed and modified the grading method previously developed in our laboratory (11, 21). The lung edema was assessed as follows: degree 0, 1, 2, and 3 for no, mild, moderate and severe edema, respectively. For inflammatory cell infiltration, the scoring was similar to the evaluation of edema extent, degree 0-3 for no, mild, moderate and severe cellular infiltration. The histopathological assessment was performed in a blind fashion by several laboratory assistants. Each one gave a score for edema and cells infiltration from 0 to 3. The individual scores for edema and cell filtration were added together to obtain a final score, ranging from 0-6.

Expression of iNOS mRNA in Lung Tissue

Reverse-transcriptase polymerase chain reaction (RT-PCR) was employed for a semiquantitative detection of iNOS mRNA in the lung tissue. We followed the procedures described in a recent study from our laboratory (31). Fresh lung tissue was snap-frozen in liquid nitrogen, and stored at -70°C for subsequent RNA extraction. Tissue was sonicated

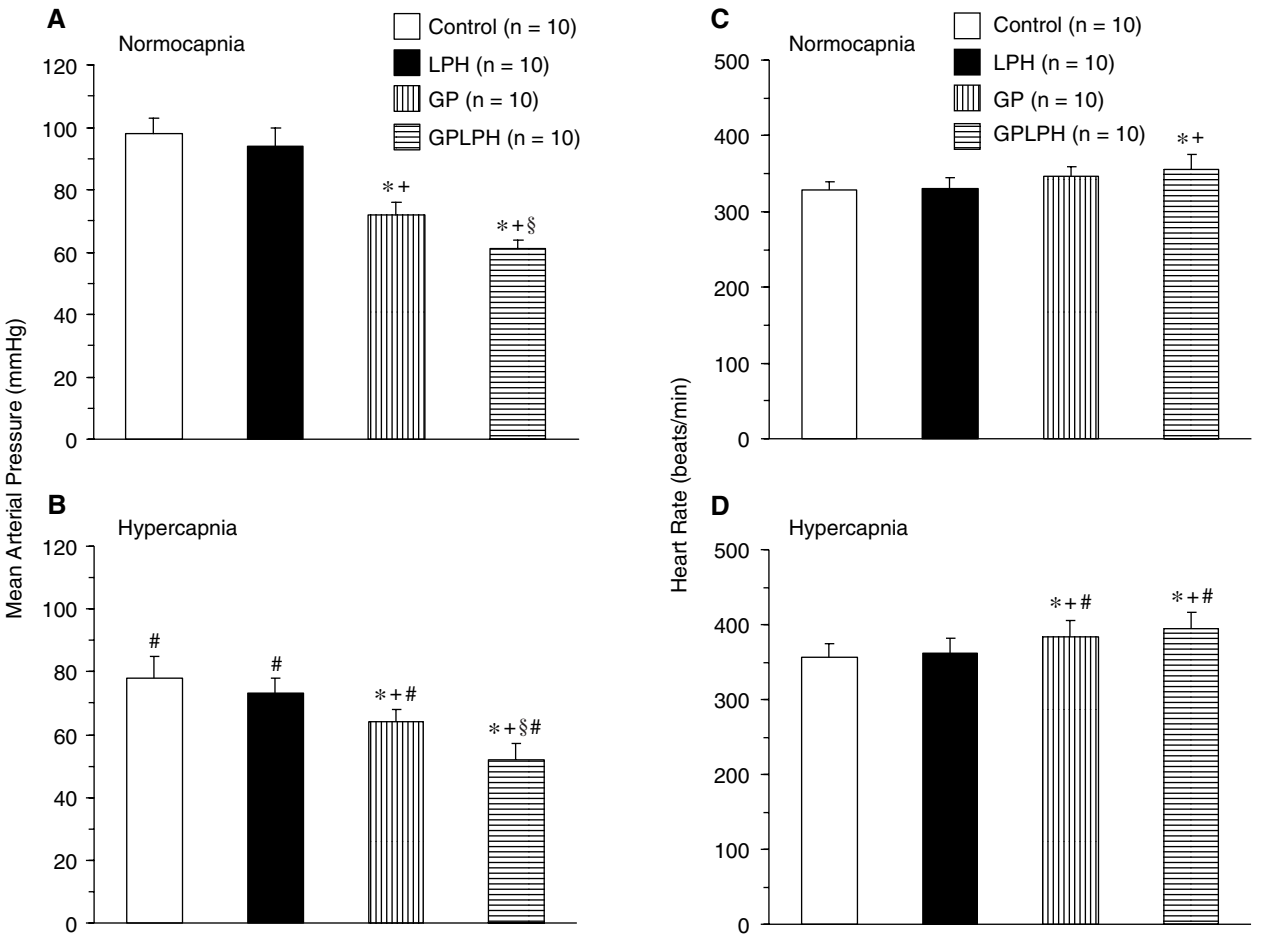

Fig. 1. Changes in mean arterial pressure (MAP, A and B), and heart rate (HR, C and D) at the end of the observation period. Intratracheal instillation of low pH saline solution (LPH) did not alter MAP. Gastric particle (GP) and GP with low pH solution (GPLPH) significantly reduced MAP. Systemic hypotension was more pronounced in GPLPH group and in hypercapnic condition. HR slightly increased in GPLPH group in normocapnic condition as well as in GP and GPLPH groups in hypercapnic condition. Values are means ± SEM. $^{*}P < 0.05$ *vs*. Control; $^{+}P < 0.05$ *vs*. LPH group; $^{\S}P < 0.05$ *vs*. GP group; and $^{\#}P < 0.05$ compared to the corresponding values in normocapnic condition. The abbreviations such as LPH, GP, GPLPH are used in figures 2-4.

under liquid nitrogen. Total RNA was extracted using Trizol reagent (Gibco BRL, Gaithesburg, MD, USA). In brief, 1 ml Trizol and 200 μl chloroform were added. The sample was centrifuged at 10,000 g for 10 min at 4°C. The supernatant was transferred to a new microcentrifuge tube which contains 500 μl isopropanol. The RNA was recovered by centrifugation for 15 min. The RNA pellets were reverse-transcribed using AMV reverse transcriptase (Superscript II; Gibco BRL, Gaithesberg, MD, USA). The product was amplified with PCR using Taq DNA polymerase and a primer pair. The reaction was performed on a PTC-200 Peltier Thermal Cycler (MJ Research, Boston, MA, USA). The primers and the size of the iNOS and glyceraldehyde phosphate dehydrogenase (GAPDH) were as follows: iNOS, 5'-CTTCAGGTATGCGGTATTGG-3' (sense), and 5'-CATGGTGAACACGTTCTTGG-3' (anti-sense) and amplified product of 351 bp; GAPDH, 5'-TCCCTCAAGATTGTCAGCAA-3' (sense), and 5'-AGATCCACAACGGATACATT-3' (anti-sense) and 308 bp. We performed PCRs for 30 cycles under the following conditions: denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and elongation at 72°C for 1 min. Band intensities of the amplified DNA were compared after visualization on a UV transilluminator. Scanning densitometry was performed with the Image Scan and Analysis System (Alpha-Innotech Corp. San Leandro, CA, USA). The relative optical density was calculated and expressed as iNOS/GAPDH.

Experimental Protocols

Gastric Acid Particles Instillation

Rats were randomly assigned to four groups

Table 1. Blood gases and ACID-base status in various groups under normocapnic and hypercapnic conditions

	pH	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	SaO_2 (%)
Normocapnia				
Control	7.24 ± 0.04	94 ± 5	38 ± 2	95.8 ± 4.3
LPH	7.10 ± 0.03*	87 ± 4*	42 ± 3*	90.2 ± 3.5*
GP	6.34 ± 0.06*+	76 ± 3*+	48 ± 5*+	81.6 ± 3.5*+
GPLPH	6.16 ± 0.08*+§	70 ± 4*+§	56 ± 4*+§	76.4 ± 2.8*+§
Hypercapnia				
Control	7.13 ± 0.03#	83 ± 4#	67 ± 3#	92.8 ± 4.1#
LPH	6.48 ± 0.05*#	75 ± 5*#	73 ± 4*#	86.2 ± 2.4*#
GP	6.02 ± 0.03*+#	64 ± 6*+#	79 ± 6*+#	62.5 ± 3.4*+#
GPLPH	5.48 ± 0.06*+§#	52 ± 3*+§#	85 ± 7*+§#	54.6 ± 2.8*+§#

Values are means ± SEM (n = 10 in each group). *$P < 0.05$ *vs*. control. +$P < 0.05$ *vs*. LPH. §$P < 0.05$ *vs*. GP. #$P < 0.05$ *vs*. the corresponding values in normocapnic condition.

(n = 10 in each group): 1) Control group: PSS 0.5 ml at pH 7.24; 2) Acid group (low pH group, LPH): PSS 0.5 ml adjusted to pH 1.25 with hydrochloric acid (HCl); 3) Gastric particle group (GP): gastric particles 40 mg/ml in 0.5 ml PSS; and 4) Gastric particle with acid group (GP low pH, GPLPH): gastric particles 40 mg/ml with 0.5 ml PSS adjusted to pH 1.25 with HCl. These agents were administered through a polyethylene catheter inserted into the endotracheal tube. The animals were observed for 6 h. To investigate the effects of hypercapnia on ALI and associated changes induced by intratracheal instillation of gastric acid particles, two series of experiments were carried out. Series one examined the changes under normocapnic ventilation (0% CO_2, 30% O_2, and 70% N_2), and series two under hypercapnic ventilation (10% CO_2, 20% O_2, and 70% N_2). Each series of experiments consisted of four groups, Control, LPH, GP, and GPLPH, for a total of eight groups.

Statistical Analysis

The data were expressed as means ± SEM. Comparisons within and among groups were made using one way analysis of variance with repeated measures, followed by a *post hoc* comparison with Newman-Keul's test. Differences were considered to be statistically significant at $P < 0.05$.

Results

Arterial Pressure and Heart Rate

Mean arterial pressure at the end of 6-h observation period was significantly reduced by intratracheal instillation of gastric particles (GP) and GP with low pH saline solution (GPLPH). Hypercapnia tended to enhance systemic hypotension (Fig. 1, A and B). Slight tachycardia was observed in GPLPH group under normocapnic condition, as well as in GP and GPLPH groups under hypercapnic condition (Fig. 1, C and D).

Blood Gases and Acid-Base Status

Arterial blood gases and acid-base status were taken 4 h after the experiment. LPH slightly but significantly decreased pH, PaO_2, and SaO_2, whereas it increased $PaCO_2$. The changes in blood gases and acid-base status were excerbated in GP and GPLPH groups and under hypercapnic condition (Table 1).

LW/BW Ratio, LW Gain, Protein Concentration in Bronchoalveolar Lavage (PCBAL) and Evans Blue Dye Leakage

Table 2 summarizes the values of LW/BW ratio, LWG, PCBAL and Evans blue dye content in the lung tissue. LPH caused slight increases in these variables. The extent of increases in these variables was aggravated by GP and GPLPH as well as in hypercapnic condition.

Plasma Nitrate/Nitrite (NOx), Methyl Guanidine (MG), Myeloperoxidase (MPO) and Phospholipase A_2 (PLA_2)

Fig. 2 and Fig. 3 illustrate the time course of changes in nitrate/nitrite, methyl guanidine, MPO and PLA_2. GPLPH, GP and LPH caused elevation of these chemical factors to different levels. It appeared that NOx, MG, MPO and PLA_2 reached a plateau 2 h after the challenge. The magnitude of increase was much greater in hypercapnic condition.

Table 2. Lung weight (LW) to body weight (BW) ratio (LW/BW), lung weight gain (LWG), protein concentration in bronchoalveolar lavage (PCBAL), and evans blue dye content in the lung tissue

	LW/BW (× 100)	LWG (g)	PCBAL (ng/dL)	Evans Blue Dye Content (ng/mg)
Normocapnia				
Control	0.48 ± 0.02	0.01 ± 0.01	20.3 ± 2.6	2.1 ± 0.3
LPH	0.74 ± 0.04*	0.24 ± 0.03*	78.4 ± 4.6*	4.8 ± 0.5*
GP	1.34 ± 0.08*+	0.98 ± 0.06*+	196.6 ± 9.8*+	10.3 ± 0.9*+
GPLPH	2.26 ± 0.12*+§	1.22 ± 0.11*+§	246.9 ± 11.4*+§	14.9 ± 1.4*+§
Hypercapnia				
Control	0.51 ± 0.03	0.02 ± 0.02	22.4 ± 2.2	2.6 ± 0.4
LPH	0.98 ± 0.06*#	0.36 ± 0.44*#	103.6 ± 5.2*#	7.9 ± 0.6*#
GP	2.33 ± 0.14*+#	1.42 ± 0.09*+#	248.4 ± 10.2*+#	22.1 ± 1.6*+#
GPLPH	4.02 ± 0.20*+§#	2.88 ± 0.16*+§#	326.8 ± 11.4*+§#	35.2 ± 2.2*+§#

Values are means ± SEM (n = 10 in each group). $^{*}P < 0.05$ *vs*. control. $^{+}P < 0.05$ *vs*. LPH. $^{\S}P < 0.05$ *vs*. GP. $^{\#}P < 0.05$ *vs*. the corresponding values in normocapnic condition.

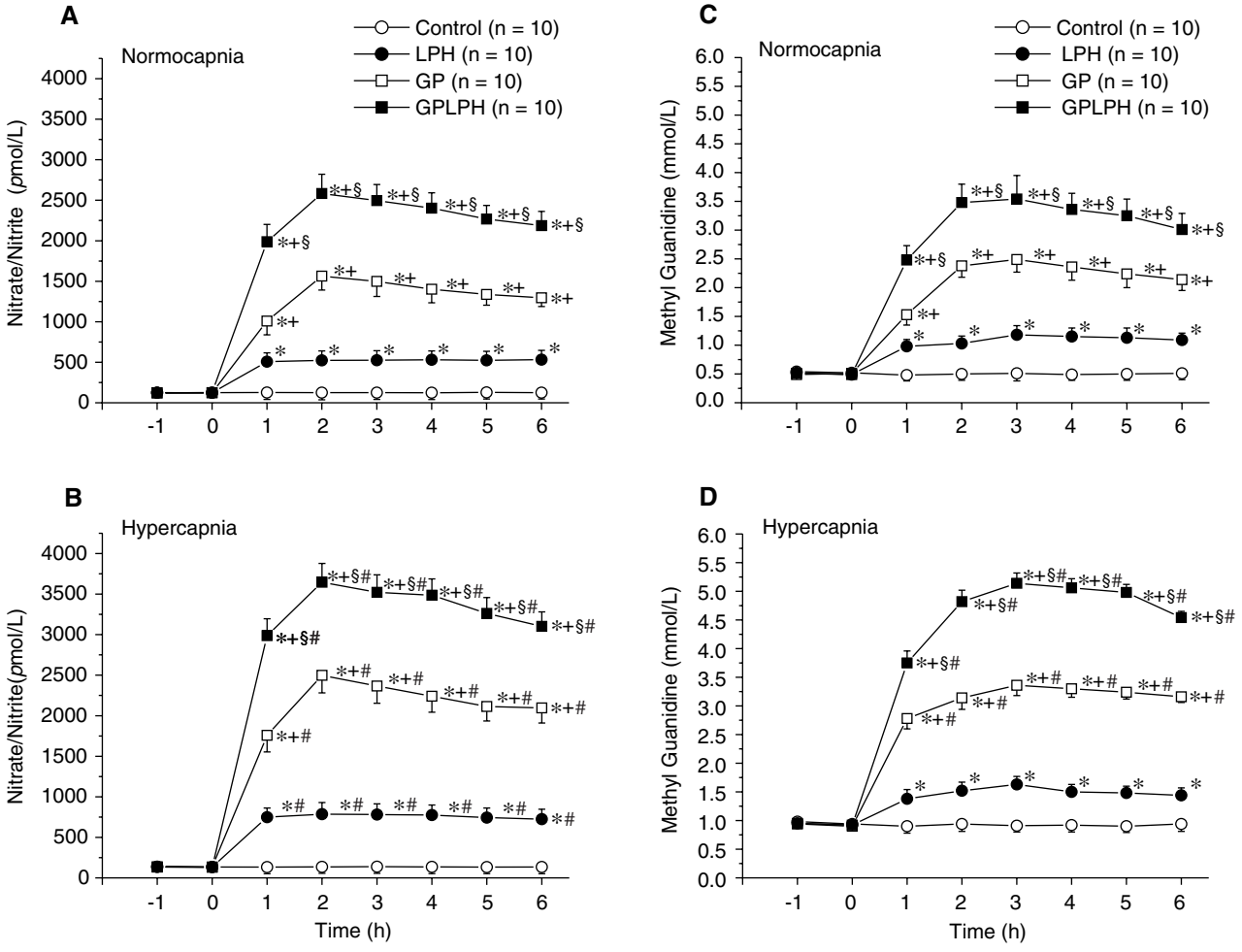

Fig. 2. Time course of changes in plasma levels of nitrate/nitrite (A, normocapnia and B, hypercapnia) and methyl guanidine (C, normocapnia and D, hypercapnia). LPH, GP and GPLPH elevated these two variables. The extent of increase was in an order of LPH, GP and GPLPH. The concentration of nitrate/nitrite in hypercapnic condition was higher than those in normocapnic condition in GP and GPLPH groups. $^{*}P < 0.05$ *vs*. Control; $^{+}P < 0.05$ *vs*. LPH group; $^{\S}P < 0.05$ *vs*. GP group; and $^{\#}P < 0.05$ compared to the corresponding values in normocapnic condition.

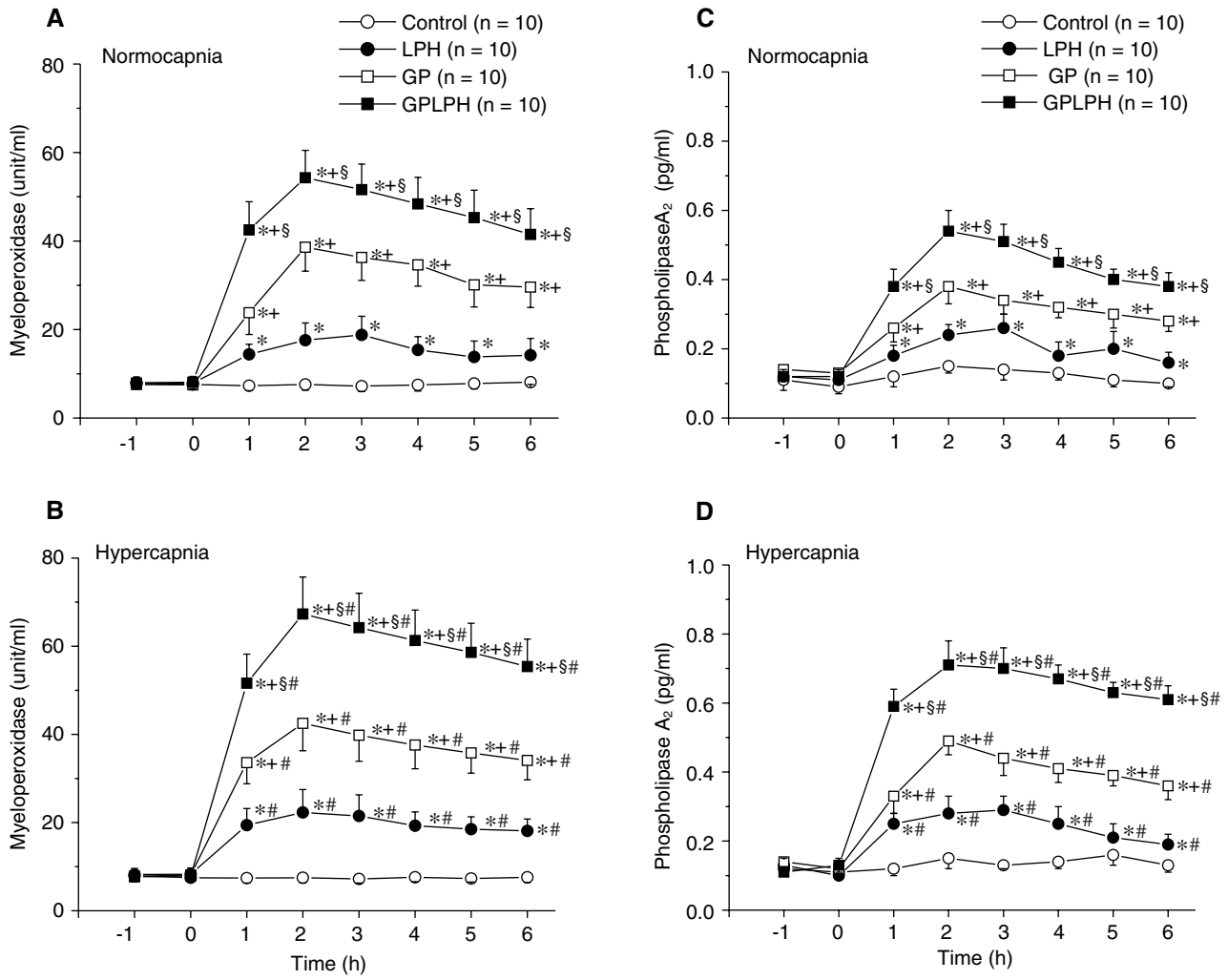

Fig. 3. Myeloperoxidase (MPO, A, normocapnia and B, hypercapnia) and phospholipase A_2 (PLA_2, C, normocapnia and D, hypercapnia). Increases in MPO and PLA_2 in various groups under normocapnic and hypercapnic conditions were similar to those in Fig. 2. Statistical symbols used were the same as those in Fig. 2.

Pro-Inflammatory Cytokines

LPH, GP, GPLPH resulted in significant increases in TNF_α, IL-1β and IL-6. In contrast to the changes in NOx, MG, MPO and PLA_2, the pro-inflammatory reached a peak at 3 h after challenge. Similarly, the increases caused by these challenges were greater in hypercapnia than those in normocapnia (Fig. 4).

Lung Pathology

The histopathological micrographys showed that intratracheal instillation of LPH, GP and GPLPH caused various degrees of lung injury. The lung pathology was characterized by edema and hemorrhage with inflammatory cell infiltration (Fig. 5). Quantitation of the lung injury indicated that LPH did not cause the lung pathology. GP and GPLPH significantly exacerbated the lung injury. The effects were more prominent in hypercapnic than in normocapnic condition (Table 3).

Expression of Inducible NO Synthase (iNOS) mRNA

Determination of iNOS mRNA expression with RT-PCR disclosed that GP and GPLPH upregulated the iNOS in normocapnic condition. Under hypercapnia, the iNOS upregulation by LPH, GP and GPLPH was more pronounced compared to those under normocapnia (Fig. 6).

Discussion

The result of our current study revealed that intratracheal instillation of gastric particles in normal or low pH saline solution resulted in systemic hypotension. These insults caused impairment in

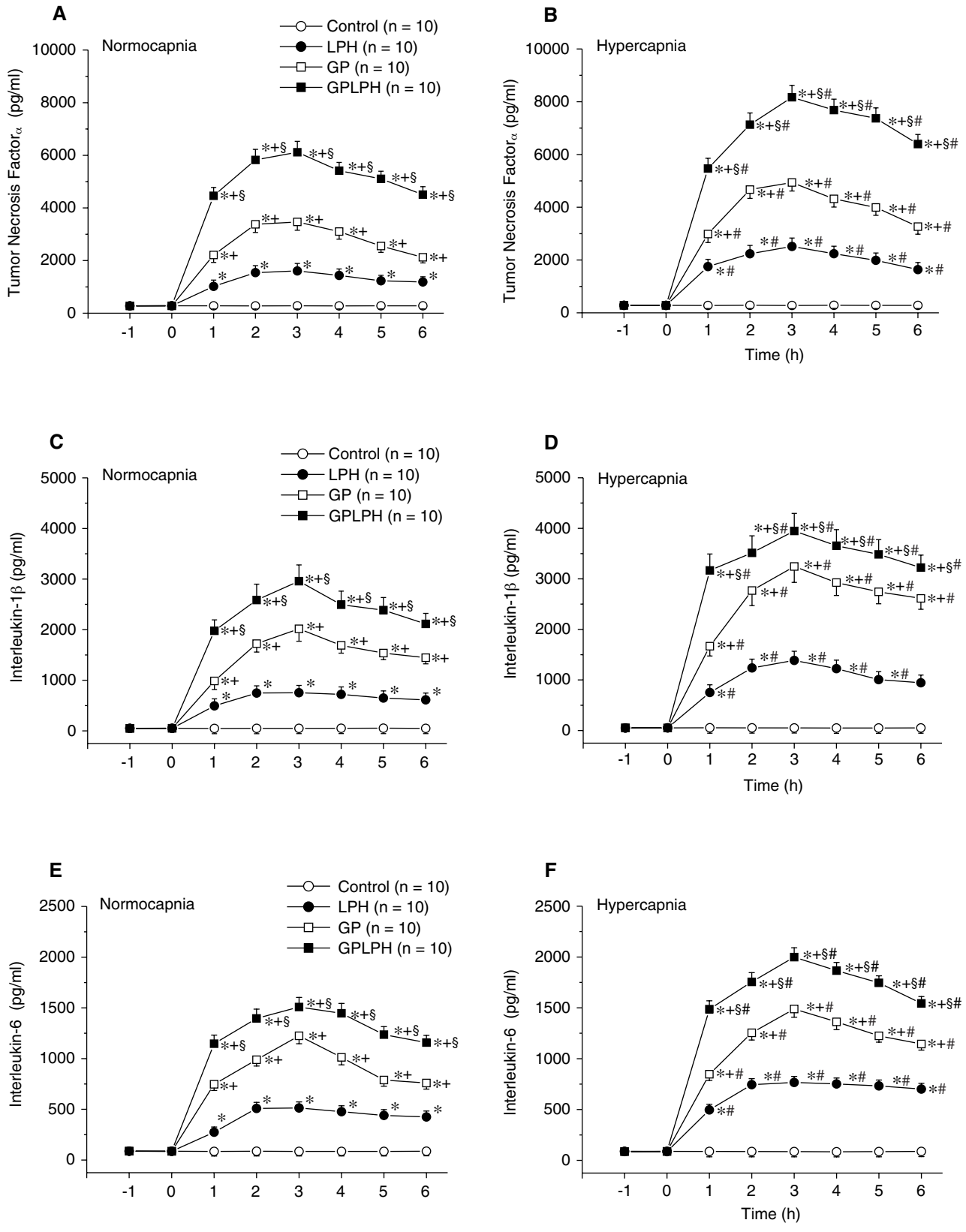

Fig. 4. Tumor necrosis factor$_\alpha$ (TNF$_\alpha$, A, normocapnia and B, hypercapnia), interleukin-1β (IL-1β, C, normocapnia and D, hypercapnia) and interleukin-6 (IL-6, E, normocapnia and F, hypercapnia). The increases in TNF$_\alpha$, IL-1β and IL-6 were in an order of LPH, GP and GPLPH. The concentration of these pro-inflammatory cytokines was higher in hypercapnic condition than in normocapnic condition in all groups except control. The statistical symbols were the same as those in Fig. 2.

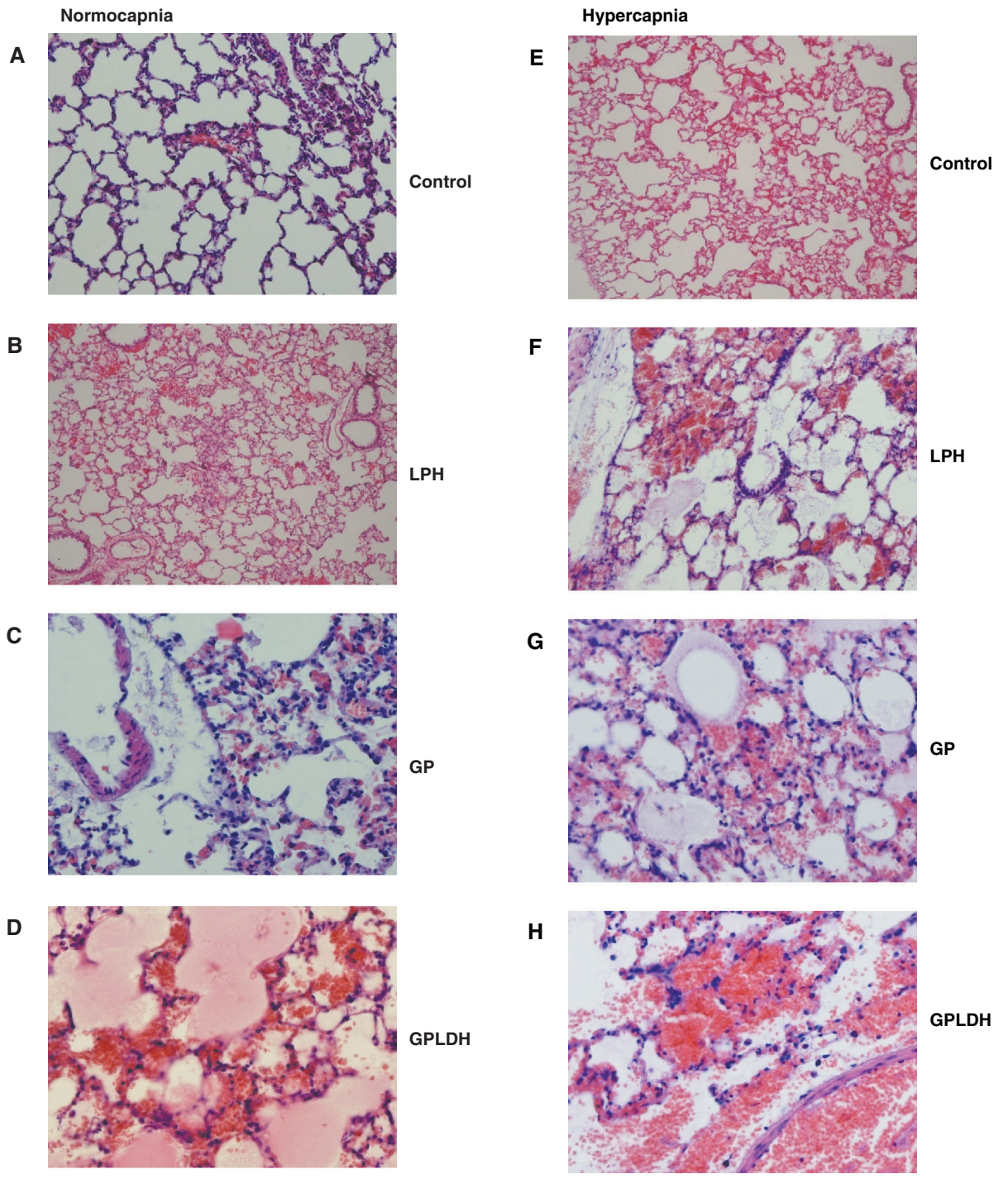

Fig. 5. Histopathological micrography of the lung in Control (A), LPH (B), GP (C) and GPLPH (D) groups in normocapnic condition, and in Control (E), LPH (F), GP (G) and GPLPH (H) under hypercapnic condition. Note the slight edematous changes in LPH groups. Moderate to severe alveolar edema and hemorrhage with infiltration of inflammatory cells in GP and GPLPH groups. The pathological changes were more pronounced in hypercapnic than in normocapnic condition.

pulmonary gas exchange and ALI as evidenced by the increases in lung weight, protein and dye leakage into the lung tissue. In addition, gastric particle instillation significantly elevated plasma nitrate/nitrite, methyl guanidine, myeloperoxidase and PLA_2. The lung pathology was characterized by alveolar edema and hemorrhage with inflammatory cell infiltration. Pro-inflammatory cytokines such as TNF_α, IL-1β and interleukin-6 significantly increased. The inducible nitric oxide synthase was upregulated. These changes

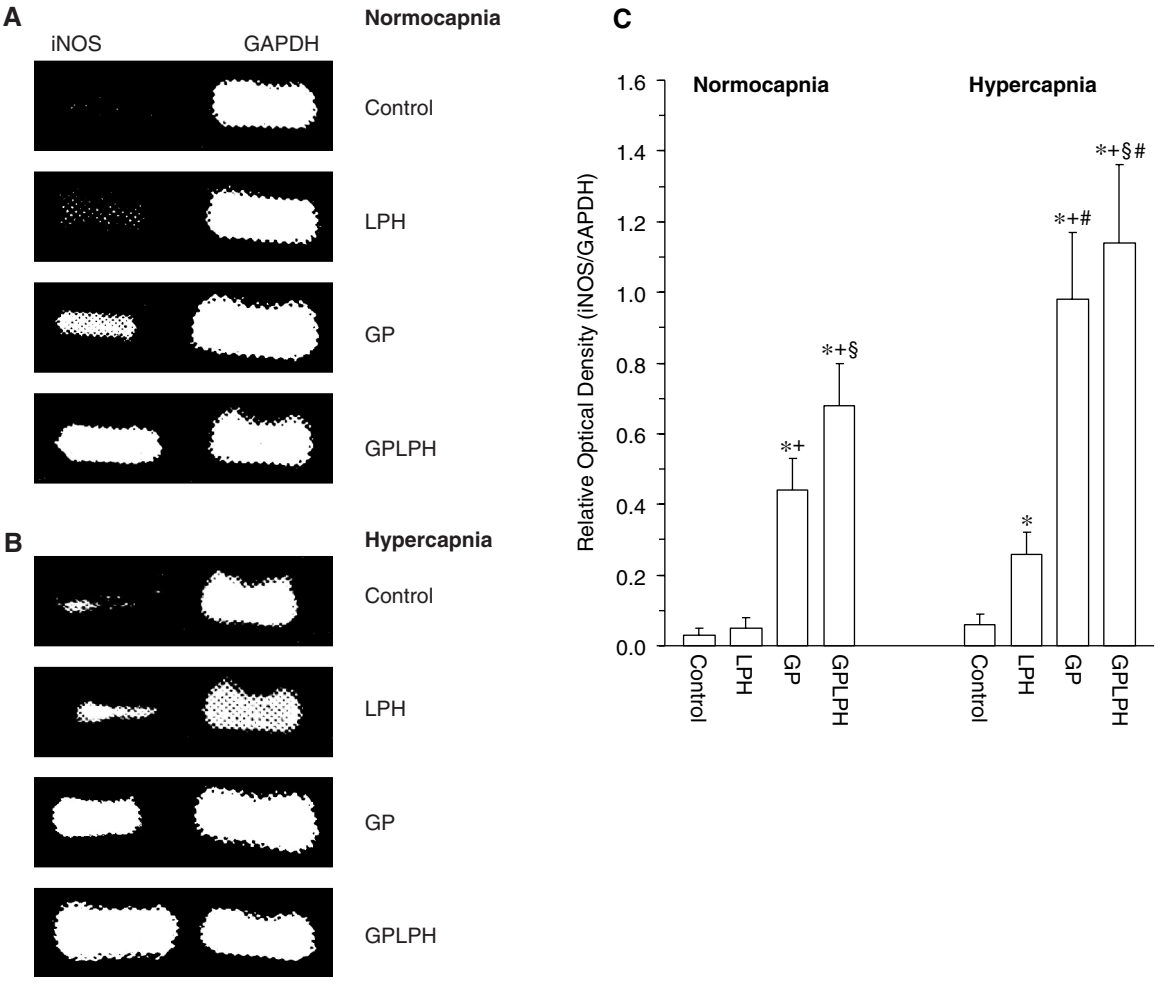

Fig. 6. Expression of inducible nitric oxide synthase (iNOS), with glyceraldehyde phosphate dehydrogenase (GAPDH) served as a contrast. The reverse-transcriptase polymerase chain reaction (A and B) and the relative optical density (C, iNOS/GAPDH ratio) indicate the enhancement of iNOS expression by LPH, GP and GPLPH under normocapnic and hypercapnic conditions.

Table 3. Lung injury score in various groups

	Lung Injury Score
Normocapnia	
Control	0.08 ± 0.03
LPH	0.12 ± 0.05
GP	$1.32 \pm 0.11^{*+}$
GPLPH	$2.18 \pm 0.15^{*+\S}$
Hypercapnia	
Control	0.11 ± 0.06
LPH	0.15 ± 0.05
GP	$2.47 \pm 0.18^{*+\#}$
GPLPH	$5.26 \pm 0.32^{*+\S\#}$

Values are means ± SEM (n = 10 in each group). $^{*}P < 0.05$ *vs*. control. $^{+}P < 0.05$ *vs*. LPH. $^{\S}P < 0.05$ *vs*. GP. $^{\#}P < 0.05$ *vs*. the corresponding values in normocapnic condition.

were more pronounced following gastric particle instillation with low pH solution. Furthermore, hypercapnia significantly enhanced pathophysiological and biochemical changes following intratracheal gastric particle instillation.

Exposure of lung tissue to acidic condition causes direct injury. Subsequemtly, inflammatory reaction leads to activation of transcriptinal factor such as nuclear factor-κB (3, 5, 32, 34). Activation and recruitment of neutrophils/macrophages are central to the cellular responses and alveolar-endothelial barrier damage (1, 44, 48, 49). The results of the present study showed that the plasm levels of pro-inflammatory cytokines (TNF_{α}, IL-1β and IL-6) reached their peaks at 2-3 h following gastric particle instillation. The involvement of pro-inflammatory chemokines and cytokines in ALI has been well documented (41, 45, 48). In addition,

oxidative stress, oxidants and reactive oxygen species are important factors in the pathogenesis of ALI and ARDS (13). We observed that plasma nitrate/nitrite, methyl guanidine (an indicator of hydroxyl radical), myeloperoxidase, and PLA_2 increased significantly in 1 h and reached high steady levels through the 6-h observation period. The increase in myeloperoxidase indicates neutrophils and macrophages recruitment (32). Secretory, cytosolic and Ca^{2+}-independent form of PLA_2 was elevated in patients with ARDS (39) and fat embolism syndrome (23). In a murine model of acute lung injury by sepsis or acid aspiration, Nagase *et al*. (37) provided evidence to suggest that cytosolic PLA_2 played a key mediator in the genesis of lung damage. *In vitro* study using cultured lung microvascular endothelial cells, treatment with secreted PLA_2 induced the expression of chemokines and adhesion molecules such as interleukin-8, growth related gene α, epithelial-neutrophil artivatung protein-78 and intercellular edhesion molecule-1 (4). PLA_2 and other cytolytic enzymes in inflammatory lung injury account for the surfactant alteration following gastric particle instillation (15).

Generation of NO through upregulation of iNOS isoform has been shown to be detrimental to the alveolar endothelial-epithelial barrier in various models of lung injury (2, 14, 19, 20, 42). Jian and coworkers (20) found that acid instilled into the rat's lung caused lung injury accompanied by increases in nuclear cell counts, neutrophil counts, albumin concentration, TNF_α, interleukin-6 and nitrate/nitrite in bronchoalveolar lavage fluid. These changes were significantly attenuated by nonspecific and specific iNOS inhibitors (N^G-monomethyl-L-arginine and ONO-1714). In the present study, increase in plasma nitrate/nitrite and iNOS upregulation were consistent with the notion that NO release *via* iNOS played a pivotal role in ALI due to acid aspiration. Furthermore, our previous studies added evidence to the detrimental role of NO/iNOS in ALI caused by enterovius infection (22), endotoxemia (11); ischemia-reperfusion (45), fat embolism syndrome (23); leptospirosis (10) and other causes.

Several reports have addressed the beneficial effects of "permissive hypercapnia" on the critically ill and in lung ventilatory strategies for the protection of acute lung injury and/or ARDS (12, 27, 28). Animal experimentations with increased ispired CO_2 to produce hypercapnic acidosis demonstrated that high $PaCO_2$ at 70 to 110 mmHg exerts protective effects on ALI induced by intratracheal lipopolysaccharide and hyperventilation (7, 26). On the contrary, hypercapnic acidosis at a lower level ($PaCO_2$ 60 mmHg and pH 7.17) induced by reduction in respiratory rate and tidal volume excerbated the pulmonary inflammatory responses following intravenous administration of lipopolysaccharide in anesthetised rabbits (30). In anesthetized rats, Laffey *et al*. (27) demonstrated the dose-dependent protective effects of hypercapnia on ALI caused by mesenteric ischemic-reperfusion. They reported that 5% CO_2 exerted a maximal effect. An increase in inspired CO_2 above 7.5% did not produce additional effect. A prospective clinical study assessing the effects of permissive hypercapnia ($PaCO_2$ 67 mmHg and pH 7.23) reported impairment of pulmonary gas exchange in patients with ARDS (17). In the present study, the $PaCO_2$ and pH were changed with combined alterations in inspired air and pulmonary gas exchange (Table 1). Our results revealed that hypercapnia worsened ALI following intratracheal instillation of gastic acid particle. Appreciating that CO_2 may modulate the chemical reactivity of nitric oxide-derived inflammatory oxidants (16, 29), the difference in $PaCO_2$ may account at least in part the discrepanany of effects of hypercapnia on ALI induced by lipopolysaccharide, hyperventilation, mesenteric ischemia-reperfusion, and acid aspiration. Hypercapnia in the presence of reactive inflammatory mediators stimulates iNOS gene expression, lung cell protein tyrosine nitration. These actions of hypercapnia without pH change cause impairment of endothehial-epithehial barrier function (29). Our data indicated that low pH saline solution (LPH) and gastic particles with low pH solution (GPLPH) enhanced pulmonary inflammatory responses leading to severer lung injury. Whether the mileu of low pH alone affected lung inflammation following acid aspiration reguires further investigation. In addition, reduction in O_2 fraction in inspired air from 30% in normocapnic ventilation to 20% in hypercapnic ventilation may have resulted in hypoxia (Table 1). Possible influence of hypoxia remains to be determined.

In summary, the current investigation in anesthetized rats revealed that inflammatory responses to acid challenge in the lung involved NO release *via* the iNOS isoform, pro-inflammatory cytokines, PLA_2 and myeloperoxidase. Hypercapnia through the change in inspired air tended to excerbate ALI and associated changes following intratracheal instillation of gastric particles.

Acknowledgments

This study was supported in part by grants from the National Science Council (NSC 95-2320-B-320-004 and NSC 95-2320-B-320-008).

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