Niacinamide Abrogates the Organ Dysfunction and Acute Lung Injury Caused by Endotoxin

Shang-Jyh Kao, MD, Demeral David Liu, DDS, PhD,†‡ Chain-Fa Su, MD,‡§ and Hsing I. Chen, MD, PhD‡¶*

Abstract: Poly (ADP-ribose) synthabse (PARS) or polymerase (PARP) is a cytotoxic enzyme causing cellular damage. Niacinamide inhibits PARS or PARP. The present experiment tests the effects of niacinamide (NCA) on organ dysfunction and acute lung injury (ALI) following lipopolysaccharide (LPS). LPS was administered to anesthetized rats and to isolated rat lungs. In anesthetized rats, LPS caused systemic hypotension and increased biochemical factors, nitrate/nitrite (NO_x), methyl guanidine (MG), tumor necrosis factor$_\alpha$ (TNF_α), and interleukin-1$_\beta$ (IL-1$_\beta$). In isolated lungs, LPS increased lung weight (LW) to body weight ratio, LW gain, protein and dye tracer leakage, and capillary permeability. The insult also increased NO_x, MG, TNF_α and IL-1$_\beta$ in lung perfusate, while decreased adenosine triphosphate (ATP) content with an increase in PARP activity in lung tissue. Pathological examination revealed pulmonary edema with inflammatory cell infiltration. These changes were abrogated by posttreatment (30 min after LPS) with NCA. Following LPS, the inducible NO synthase (iNOS) mRNA expression was increased. NCA reduced the iNOS expression. Niacinamide exerts protective effects on the organ dysfunction and ALI caused by endotoxin. The mechanisms may be mediated through the inhibition on the PARP activity, iNOS expression and the subsequent suppression of NO, free radicals, and proinflammatory cytokines with restoration of ATP.

Key Words: free radical, nitric oxide, inducible nitric oxide synthase, poly (ADP-ribose) polymerase, proinflammatory cytokines

(*J Cardiovasc Pharmacol*™ 2007;50:333–342)

INTRODUCTION

Niacinamide is a compound of the soluble B complex. It exerts inhibitory effects on the poly (ADP-ribose) synthase (PARS) or poly (ADP-ribose) polymerase (PARP). The nuclear enzyme can be activated by strand breaks in DNA caused by reactive oxygen species and peroxynitrite.[1,2] PARP is cytotoxic by massive depletion of intracellular nicotinamide adenine dinucleotide (NAD^+) and adenosine triphosphate (ATP). Inhibition of PARP activity reduces the ischemia-reperfusion injury of the heart, skeletal muscle, and brain.[3–5] In addition, PARP inhibition with a specific blocker, 3-aminobenzamide attenuates the acute lung injury induced by endotoxin.[6]

The inhibitory effects of niacinamide or its related substances, nicotinamide and nicotinic acid, on the PARP activity are protective to cell damage caused by oxidative stress.[7–10] Our laboratory has reported that PARS or PARP inhibition with niacinamide attenuates the ischemia-reperfusion hepatic injury.[11]

Previous and recent studies from our laboratory have investigated the pathogenic mechanisms and potential therapeutic regimen on the organ dysfunction and acute lung injury (ALI) induced by endotoxin.[12–16] The present study was designed to evaluate the protective effect of niacinamide on organ dysfunction and ALI caused by endotoxin in anesthetized rats and isolated perfused lungs. We also aimed to elucidate the possible mechanisms of the protective role of niacinamide on the biochemical factors for organ function and lung changes. Our results indicate that niacinamide mitigates the multiple organ dysfunction and ALI induced by endotoxin.

MATERIALS AND METHODS

Experimental Models

We used male Spague-Dawley (SD) rats (age, 12 to 15 weeks; weight 330 to 360 g) obtained from the National Animal Center and housed in the University Laboratory Animal Center with adequate environmental 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.

Acute experiments were carried out in anesthetized rats and isolated perfused rat's lungs. Rats were anesthetized with an intraperitoneal (IP) injection of sodium pentobarbital (40 mg/kg). The femoral artery and vein were cannulated. The arterial catheter was connected to a pressure transducer to monitor the arterial pressure (AP) and heart rate (HR) on a polygraph recorder (Power Lab, AD Instruments, Mountain

Received for publication March 7, 2007; accepted May 7, 2007.

From the *Division of Chest Medicine, Internal Medicine, Shin-Kong Wu-Ho-Su Memorial Hospital, School of Respiratory Therapy, Taipei Medical University, and College of Medicine, Fu-Jen University, Taipei; †Department of Dentistry; ‡Neuro-Medical Scientific Center; §Department of Neurosurgery; and ¶Institute of Integrative Physiology and Clinical Sciences, Tzu Chi Hospital and University, Hualien, Taiwan.

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

The authors state that they have no financial interest in the products mentioned within this article.

Reprints: Hsing I. Chen, MD, PhD, Institute of Integrative Physiology and Clinical Sciences, Tzu Chi University, No. 701, Sec. 3, Jhongyang Rd., Hualien 97004, Taiwan (e-mail: chenhi@mail.tcu.edu.tw).

View, CA). The venous catheter was used for administration of supplemental anesthetics, drugs or fluid, and collection of blood samples. The rats were intubated with an endotracheal tube to provide ventilation of 95% room air-5% CO_2 with a rodent ventilator. The respiratory rate and tidal volume were 60 to 65 breaths/min and 2 to 3 mL, respectively. The arterial pH, O_2, and CO_2 (PaO_2 and $PaCO_2$) were determined with a pH and blood gas analyzer (178 pH/Blood Gas Analyzer; Corning, Essex, UK). The pH, PaO_2, and $PaCO_2$ were maintained at 7.38 ± 0.02, 94.6 ± 2.4 mm Hg, and 37.9 ± 1.6 mm Hg, respectively, by adjusting the ventilatory rate and tidal volume.

Isolation and Perfusion of the Lungs

Isolated and perfused lungs were prepared according to the procedures described in previous studies.[17,18] A vertical incision was made along the midline of the thorax. Heparin (2 IU/g body weight) was then injected into the right ventricle. An afferent silicon catheter was inserted into the pulmonary artery through the right ventricle. An efferent catheter was installed into the left atrium via the left ventricle. The pulmonary trunk and the aorta were tied. A third ligature was placed at the atrioventricular junction to prevent the perfusate from entering into the ventricle. The isolated lungs were perfused with Krebs-Henseleit balanced solution with 6% albumin. The perfusion system included a venous reservoir and a roller pump. The venous outflow was diverted via the efferent catheter into the reservoir. The latter was placed in a 38° water bath for constant temperature. Pulmonary arterial pressure (PAP) and venous pressure (PVP) were measured from sideports in the afferent and efferent catheters. The lungs were perfused at constant flow (10 to 14 mL/min). Flow rate was adjusted to maintain the initial PAP at 15 to 16 mm Hg.

The isolated perfused lungs were left in situ, and the rat was placed on an electronic balance. The digital signals of the electronic balance were converted to analog signals by a digital-analog converter and recorded on a polygraph recorder. Weight changes were precalibrated on the electronic balance before preparation for the experiment. In this isolated lung in situ preparation, the changes in body weight (BW) reflected the lung weight (LW) changes.[17,18]

Lung Weight (LW) and LW Gain (LWG)

The initial LW was estimated from an equation relating to the body weight (BW).[12,14]

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

LWG was obtained from the increase in LW and also calculated as:

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

Protein Concentration in Bronchoalveolar Lavage (PCBAL)

After the experiment, lungs were lavaged twice with saline (2.5 mL per lavage). Lavage samples were centrifuged at 1500 × *g* at room temperature for 10 min. The PCBAL was determined with a spectrophotometer by measuring the change in absorbance at 630 nm after the addition of bromocresol green.[18]

Evans Blue Leakage

Five minutes before the end of experiment, Evans blue dye was injected intravenously (1 mg/kg) or added into the lung perfusate. The dye concentration in lung tissue was determined spectometrically as described by Thurston et al.[19]

Blood Sample Analysis

Blood samples (0.5 mL) were taken 1 h before LPS and 0.5, 1, 2, 3, 4, 5, and 6 h after LPS administration. Hematocrit, white blood cells, blood urea nitrogen (BUN), creatinine (Cr), glutamic oxaloacetic transaminase (GOT), and glutamic pyruvate transaminase (GPT) were determined with an autoanalyzer (Vitros 750; Johnson & Johnson, Rochester, NY).

Nitrate/Nitrite, Methyl Guanidine, Tumor Necrosis Factor$_\alpha$, and Interleukin-1$_\beta$

Blood samples or lung perfusate (0.5 mL) were taken 1 h before and 0.5, 1, 2, 3, 4, 5, and 6 h after endotoxin administration. The samples were centrifuged at 3000 × *g* for 10 min. The supernatant was used for nitrate/nitrite measurement with high-performance liquid chromatography (ENO-20; AD Instruments, Mountain View, CA).[20,21] The formation of methyl guanidine (MG) has been identified as an index of hydroxyl radical production.[22] It was determined with its fluorescence spectrum (Jasco 821-FP; Spectroscopic, Tokyo, Japan). The emission maximum was set at 500 nm and the excitation maximum at 398 nm. The assay was calibrated with authentic MG (Sigma M0377). Tumor necrosis factor$_\alpha$ (TNF_α) and interleukin-1$_\beta$ (IL-1$_\beta$) 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).[23] All agents, samples, and working standards were prepared in room temperature according to the manufacturer's directions. The optical density was measured at 450/540 nm wavelengths by automated ELISA readers.

Microvascular Permeability (K_{fc})

K_{fc} or capillary filtration coefficient as an index of microvascular permeability was calculated from the increase in LW produced by an elevation in PVP. The K_{fc} was defined as the initial weight gain rate (g/min) divided by PVP (10 cm H_2O) and LW, and expressed as g/min/cmH_2O/100 g. During the experiment with isolated lungs, PVP was elevated rapidly by 10 cm H_2O for 7 min to measure K_{fc}. The hydrostatic challenge elicited a biphasic increase in LW: an initial rapid component followed by a slow and steady component. The slow component of the weight gain was plotted on a semilog scale as a function of time. The capillary filtration rate was obtained by extrapolating the slow component of the weight gain back to zero time.[17,18]

ATP Content

Lungs were harvested after the experiments. A BioOrbit ATP Assay kit (Bio-Orbit Oy, Turku, Findland) was used to determine the lung ATP content with bioluminescence technique. The assay was based on quantitative measurement of a stable level of light as a result of an enzyme reaction catalyzed by luciferase. Under the effect of luciferase, the

luminescence evoked by interaction of ATP and luciferin was recorded photometrically after amplification by a photomultiplier. The sensitivity of ATP was in nonomolar range. We used the luciferin-luciferase reagent according to manufacturer manual. ATP served as the standard. The test procedures were described previously.[11]

PARP Activity

PARP activity in the harvested lung tissue was measured with a commercially available assay kit (Genzyme Diagnostics, Cambridge, MA). Lung tissue samples were placed on ice in 2 mL of buffer containing 50 mM Tris·Cl (pH 8.0), 25 mM $MgCl_2$, and 0.1 mM phenylmethylsulfonyl fluoride. The samples were homogenized for 30 s and then sonicated for 20 s using an ultrasonic homogenizer. The suspension was centrifuged at 3000 × *g* for 5 min at 4°C. Supernatant containing 20 μg protein, PARS buffer, 1 mM NAD, 2 μCi ^{32}P-labeled NAD (1 μCi/μL) and distilled water was mixed in a microcentrifuge tube. The reaction was allowed to proceed at room temperature for 1 min and stopped by adding 900 μL of TCA. Enzyme activity was determined by measuring the incorporation of radiolabeled NAD as PARP catalyzed the poly (ADP) ribosylation of proteins. The labeled ADP was determined by scintillation counting after TCA precipitation onto a filter. The procedures and calculation of PARP activity were according to those described by Pulido et al.[24]

Inducible and Endothelial NO Synthases (iNOS and eNOS) mRNA

Reverse-transcriptase polymerase chain reaction (RT-PCR) was employed for a semiquantitative detection of iNOS and eNOS mRNA expression in the lung tissue. Total cellular RNA was extracted from each tissue section after the experiment by the Trizol procedure (Gibco BRL, Gaithesburg, MD). Primers specific for iNOS (sense: 5′-CTTCAGG TATGC-GGTATTGG-3′ antisense: 5′-CATGGTGAACACG TTCTTGG-3′), eNOS (sense: 5′-AGCTGGCATGGGCAA CTTGAA-3′ antisense: 5′-CAGCACATCAAAGCGGCCA TT-3′), and glyceraldehyde phosphate dehydrogenase (GAPDH) (sense: 5′-TCCCTCAAGAT- TCTCAGCAA-3′ antisense: AGATCCACAACGGATACATT-3′) were used. Scanning densitometry was performed with Image Scan & Analysis System (Alpha-Innotech Corp., San Leandro, CA). We followed the procedures described in relevant studies[25,26] and manufacturer direction. The expression of GAPDH was used as an internal control.

Lung Pathology

After the LW was obtained, the lung tissue was taken for histological examination. It was immersed in 10% formaldehyde fixative for 24 h and was rinsed with tap water to remove formaldehyde. For light microscopic examination, lung tissue was dehydrated with graded alcohol and then embedded in paraffin at 60°C. A series of microsections (5 μM) was stained with hematoxylin and eosin. For quantitation of lung injury score (LIS), we employed and modified the scoring system from recent studies in our laboratory.[14,18] The LIS was assessed as follows: degree 0, no edema; degree 1, mild edema; degree 2, moderate edema; and degree 3, severe edema, various degree were scored from 0 to 3. For inflammatory cell infiltration, the grading was similar to the extent of edema (ie, degree 0, 1, 2, and 3 for no, mild, moderate, and severe cellular infiltration, respectively). The pathological assessment was performed in a blind fashion by several laboratory assistants. Each one gave a score for edema and inflammatory cell infiltration from 0 to 3. The individual scores for edema and cell aggregation were added together to obtain a total score, ranging from 0 to 6.

Endotoxin Administration

Previous studies from our laboratory have indicated that an intravenous (IV) injection of lipopolysaccharides (LPS, *Escherichia coli*; Sigma, St. Louis, MO) at a dose of 10 mg/kg produced maximal effect on the extent of ALI.[12,13] Accordingly, endotoxemia was induced by an IV injection of 10 mg/kg LPS. LPS was dissolved in PSS before use. In isolated perfused lungs, LPS (10 mg/kg body weight) was added into the venous reservoir. Each group of anesthetized rats and isolated perfused lungs were observed for 6 h.

Experimental Protocols

Thirty anesthetized rats were randomly assigned to receive vehicle (PSS), LPS, or LPS with NCA. Thirty isolated lung preparations were subjected to vehicle, LPS with NCA, or LPS without NCA posttreatment. In anesthetized rats, we tested the effects of niacinamide (200 mg/kg, IV) on the endotoxin-induced changes during the 6-h observation period. Parameters included AP, HR, biochemical factors, nitrate/nitrite, MG, TNF_α, IL-1_β, ATP, PARP activity, and iNOS as well as eNOS mRNA. In isolated perfused lungs, niacinamide (100 mg/kg body weight) was added into the venous reservoir. We obtained lung weight changes, PCBAL, Evans blue content, K_{fc}, and PAP. Nitrate/nitrite, MG, TNF_α and IL-1_β in lung perfusate, and ATP, PARP activity, NOS mRNA in lung tissue were determined. Similar changes in parameters or factors in the anesthetized rats and isolated lungs were pooled together. Niacinamide was purchased from Sigma Chemicals (St. Louis, MO). It was dissolved in PSS before use. Vehicle groups received PSS. Niacinamide (NCA) was given 30 min after endotoxin administration. The doses of NCA administration in anesthetized rats and isolated lungs were in the range used in previous studies.[11,24,27,28]

Data Analysis

Data were expressed as means ± SEM. Comparisons within and among groups were made with 1-way analysis of variance with repeated measures followed by post hoc comparison with Newman-Keul test. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Arterial Pressure and Heart Rate

Figure 1 depicts the time course of changes in mean arterial pressure (MAP, panel A) and heart rate (HR, panel B) following LPS administration in anesthetized rats. LPS caused decrease in MAP with increase in HR. Niacinamide (NCA)

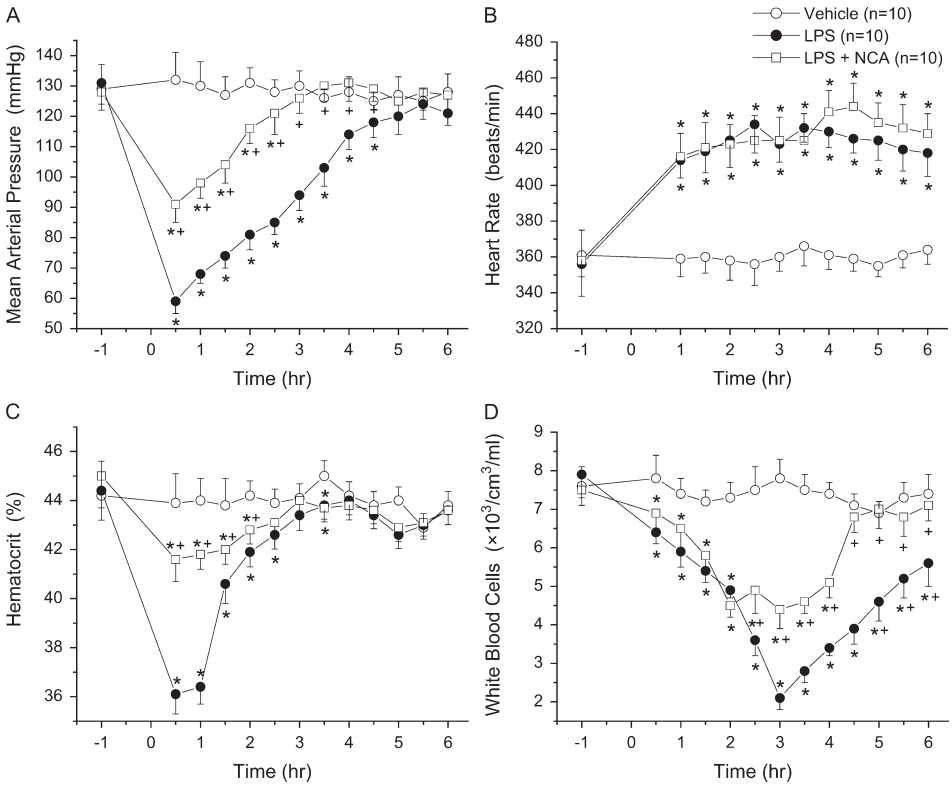

FIGURE 1. Time course of changes in mean arterial pressure (A), heart rate (B), hematocrit (C), and white blood cells (D) in anesthetized rats received vehicle, lipopolysaccharide (LPS), and LPS with niacinamide (NCA) posttreatment. The LPS-induced systemic hypotension was significantly attenuated by NCA. The tachycardia was not affected. LPS caused severe erythrocytopenia at 0.5 and 1 h and leukocytopenia at 3 h. Posttreatment with NCA attenuated the changes in blood elements. Data are means ± SEM. (vertical bar). *$P < 0.05$ compared with vehicle group; +$P < 0.05$ compared with LPS group.

posttreatment attenuated the systemic hypotension but did not affect the tachycardia.

Hematocrit and White Blood Cells

LPS resulted in decreases in hematocrit and white blood cells (Figure 1, C and D). The maximal erythrocytopenia occurred at 0.5 and 1 h, and leukocytopenia at 3 h. NCA significantly ameliorated the changes in hematocrit and white blood cells.

Blood Urea Nitrogen, Creatinine, Glutamic Oxaloacetic Transaminase, and Glutamic Pyruvate Transaminase

LPS greatly increased these biochemical factors. Posttreatment with NCA attenuated the biochemical changes (Figure 2).

Nitrate/Nitrite, Methyl Guanidine, Tumor Necrosis Factor$_\alpha$, and Interleukin-1$_\beta$

The plasma NO metabolites, hydroxyl radical, and proinflammatory cytokines were increased remarkably following LPS. NCA posttreatment abrogated the LPS-induced changes. In particular, the nitrate/nitrite and interleukin-1$_\beta$ were reduced to levels close to the basal values obtained in vehicle group (Figure 3). In isolated lungs, LPS similarly caused marked increases in these factors in the lung perfusate. Posttreatment with NCA abrogated the changes (Figure 4).

Lung Weight Changes, Pulmonary Arterial Pressure, and Microvascular Permeability (K_{fc})

In isolated lungs, LPS caused increases in lung weight (LW)/body weight (BW) ratio, lung weight gain (LWG), and K_{fc}. The changes in lung weight and capillary filtration coefficient were reduced by NCA posttreatment. Pulmonary arterial pressure (PAP) was essentially not affected by LPS. NCA, however increased PAP (Figure 5).

Protein Concentration in Bronchoalveolar Lavage (PCABL), Evans Blue Dye, ATP Content, and PARP Activity in Lung Tissue

LPS administration in anesthetized rats and in isolated lungs resulted in protein and dye leakage as well as decrease in ATP content and increase in PARP activity. NCA reduced the protein, dye leakage, and PARP activity, while restored the ATP content (Figure 6).

Expression of Inducible and Endothelial Nitric Oxide Synthases (iNOS and eNOS) mRNA in Lung Tissue

Figure 7 shows the expression of iNOS and eNOS mRNA in lung tissue following various treatments. Table 1 summarizes the values of iNOS/GAPDH and eNOS/GAPDH ratio. The data indicated that LPS in anesthetized rats and isolated lungs caused a large increase in iNOS mRNA expression and a slight increase in eNOS mRNA expression. These effects were diminished by posttreatment with NCA.

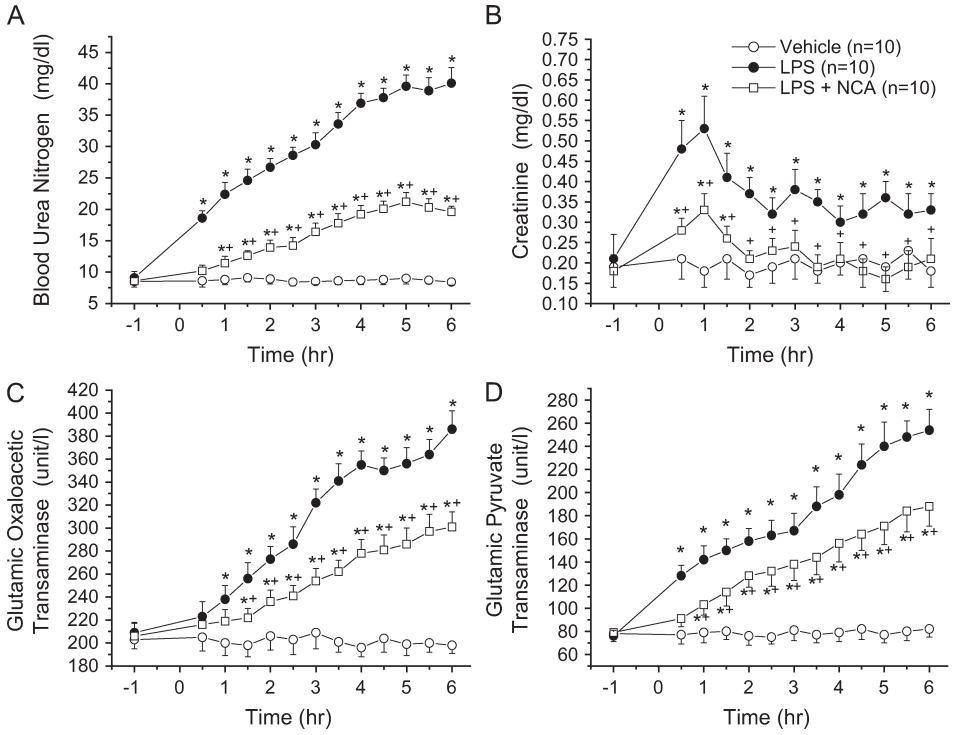

FIGURE 2. Blood urea nitrogen (A), creatinine (B), glutamic oxaloacetic transaminase (C), and glutamic pyruvate transaminase (D). Posttreatment with NCA significantly ameliorated the increases in these biochemical factors following LPS in anesthetized rats. Data are means ± SEM. *$P < 0.05$ versus vehicle group; +$P < 0.05$ versus LPS group.

Lung Injury

Histopathological microphotographs show alveolar edema and/or hemorrhage with infiltration of inflammatory cells (Figure 8). Posttreatment with NCA attenuated the lung pathological changes. The calculated lung injury score indicated that NCA mitigated the acute lung injury caused by LPS in anesthetized rats and isolated lungs (Table 2).

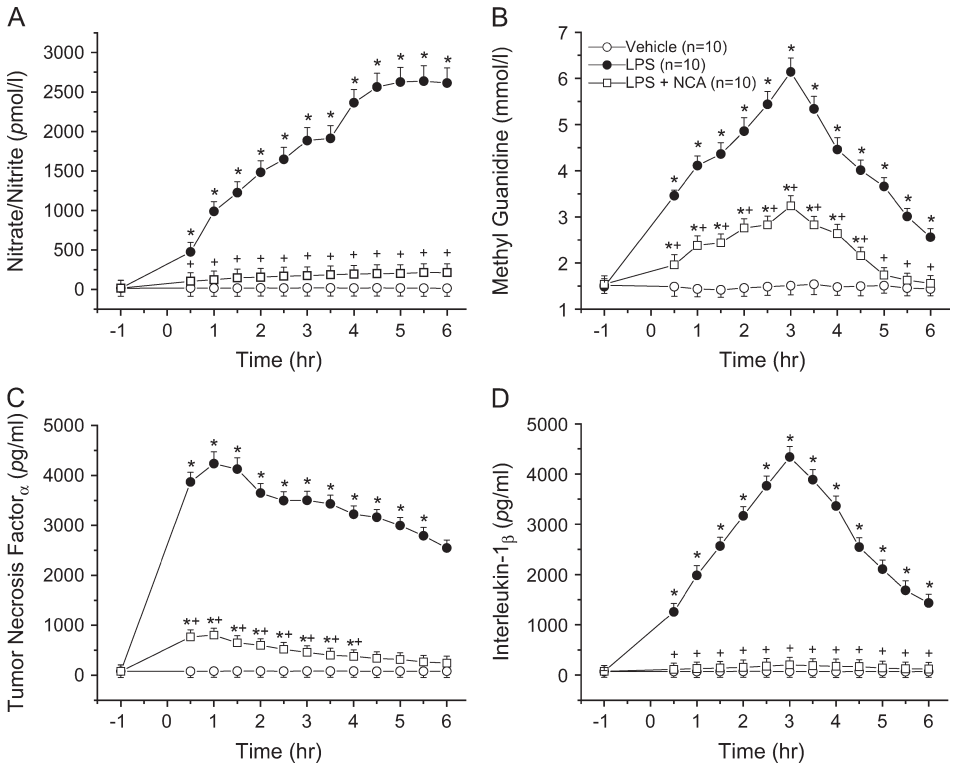

FIGURE 3. Plasma concentration of nitrate/nitrite (A), methyl guanidine (B), tumor necrosis factor-α (C), and interleukin-1β (D). NCA posttreatment reduced the LPS-induced increases in the NO metabolites, hydroxyl radical, and proinfalmmatory cytokines. Data are means ± SEM. *$P < 0.05$ versus vehicle group; +$P < 0.05$ versus LPS group.

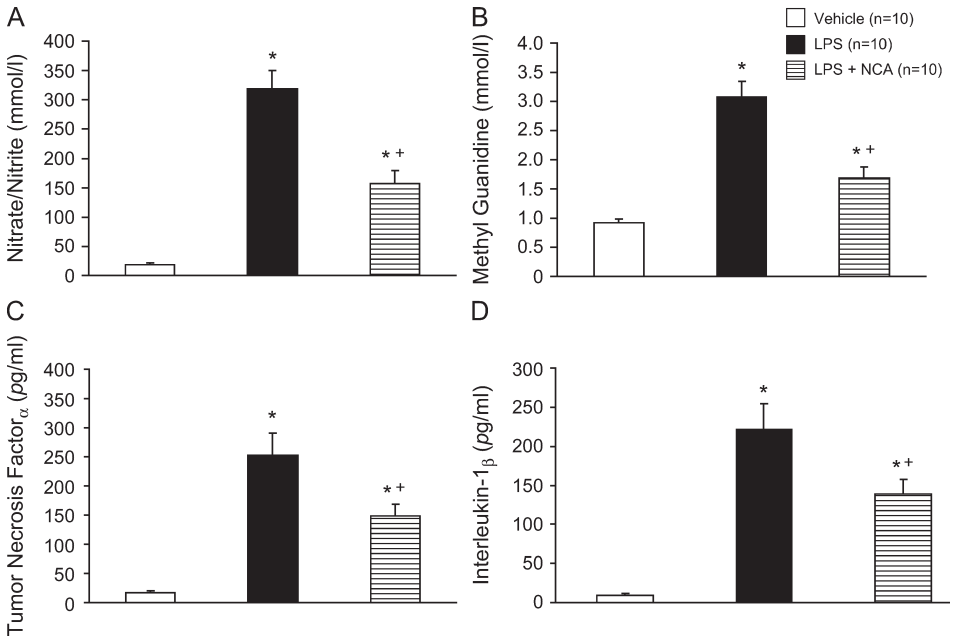

FIGURE 4. The concentration of nitrate/nitrite (A), methyl guanidine (B), tumor necrosis factor-α (C), and interleukin-1β (D) in lung perfusate from isolated perfused lungs subjected to LPS with and without NCA posttreatment. LPS elevated the NO metabolites, hydroxyl radical, and proinflammatory cytokines. NCA attenuated these changes. *$P < 0.05$ versus vehicle group; +$P < 0.05$ versus LPS group.

DISCUSSION

Previous studies have shown that PARS or PARP inhibition with 3-aminobenzamide (10 mg/kg, IP or IV injection) and/or niacinamide (50 to 750 mg/kg, IV administration) reduces the inflammatory responses to zymosan,[27] improves the impairment of endothelium-dependent and -independent pulmonary vasorelaxation,[24] abrogates the hepatic injury caused by ischemia and reperfusion,[11] and decreases the cerebral infarct volume after middle cerebral artery occlusion in hypertensive, diabetic, and mondiabetic rats.[28]

In the present investigation, we used endotoxin to induce biochemical changes for organ functions and acute lung injury (ALI) in anesthetized rats and isolated perfused rat's lungs. We found that niacinamide (NCA), exerted protective effects on the organ dysfunction and ALI following endotoxemia. This agent attenuated the LPS-induced systemic hypotension, erythrocytopenia, and leukocytopenia, but it did not affect the tachycardia. It also mitigated the biochemical changes and reduced the plasma nitrate/nitrite, methyl guanidine, tumor necrosis factor$_\alpha$ and interteukin-1$_\beta$. In isolated lungs,

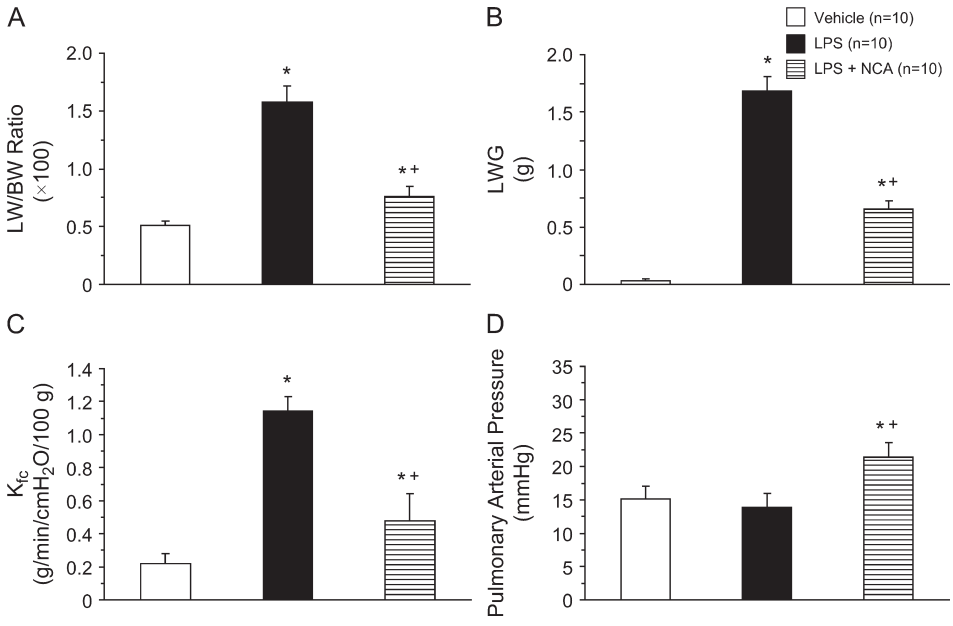

FIGURE 5. The maximal values of lung weight (LW) to body weight (BW) ratio (LW/BW, A), LW gain (LWG, B), microvascular permeability (K_{fc}, C), and pulmonary arterial pressure (PAP, D) in isolated lungs subjected to LPS with and without niacinamide (NCA) posttreatment. LPS increased the lung weight changes. The increases were abrogated by NCA posttreatment. LPS slightly, but not significantly decreased the PAP. NCA increased the PAP following LPS. *$P < 0.05$ versus vehicle; +$P < 0.05$ versus LPS.

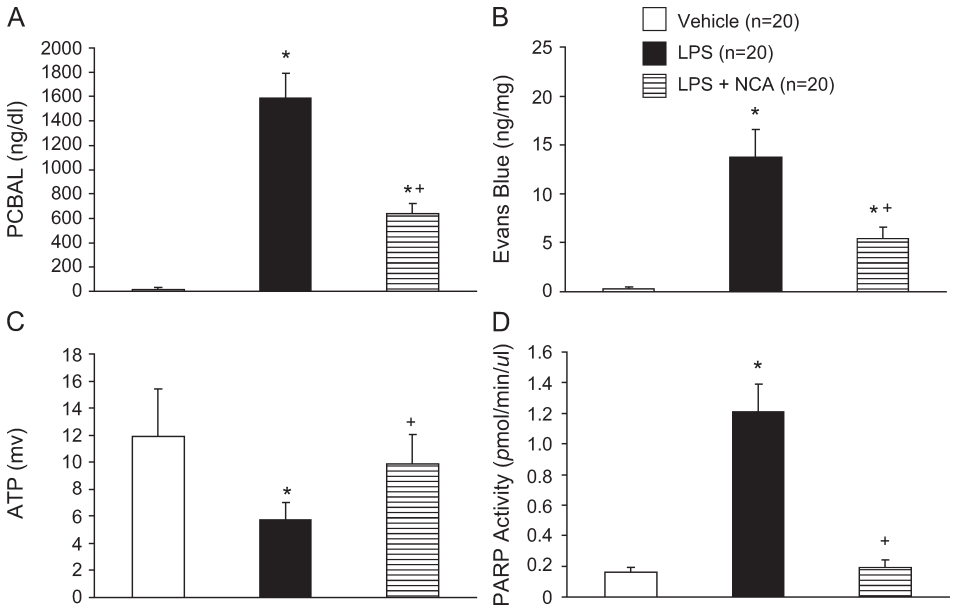

FIGURE 6. Protein concentration in bronchoalveolar lavage (PCBAL, A), Evans blue (B), adenosine triphosphate (ATP) (C) content, and PARP activity (D) in lung tissue. LPS in anesthetized rats and in isolated lungs increased the PCBAL, Evans blue dye leakage, and PARP activity and decreased the ATP content. NCA diminished the protein and dye leakage as well as PARP activity, but it restored the ATP content. (The data of LPS in anesthetized rats and isolated lungs are pooled.) $*P < 0.05$ versus vehicle; $+P < 0.05$ versus LPS group.

niacinamide decreased the lung weight changes, microvascular permeability, protein, and dye leakage. In addition, NCA resulted in reduction of NO metabolites, hydroxyl radical, and proinflammatory cytokines in the lung perfusate after LPS. LPS in anesthetized rats and isolated lungs enhanced PARP activity, NOS mRNA expression (iNOS, in particular), and decreased ATP content. This vitamin B—related agent reduced PARP and iNOS and restored ATP. A previous study from our laboratory has revealed the beneficial actions of NCA on the I/R hepatic injury.[11] We further demonstrated the protective role of NCA on the organ dysfunction and ALI induced by endotoxin.

Pharmacological inhibition and gene knockout mutant of PARS and/or poly(ADP-ribose) polymerase (PARP) have become a new approach for the experimental therapy of various disorders such as shock, stroke, ischemia/reperfusion heart and gut injury, diabetes mellitus, lung injury, and others.[29–32] Activation of PARS and/or PARP produces cytotoxicity and subsequent cell death and organ dysfunction.[33] We demonstrated in the present study that posttreatment with niacinamide abrogated the changes in biochemical factors for organ function and ALI induced by endotoxin in anesthetized rats and/or isolated lungs. The mechanisms are likely through PARS and/or PARP inhibition. The mitochondrial respiration in cultured endothelial cells was severely depressed by serum from septic patients. Addition of NG-methyl-L-arginine (an NOS inhibitor) and 3-aminobenzamide (a specific PARP blocker) significantly attenuated the suppression of mitochondrial function.[34]

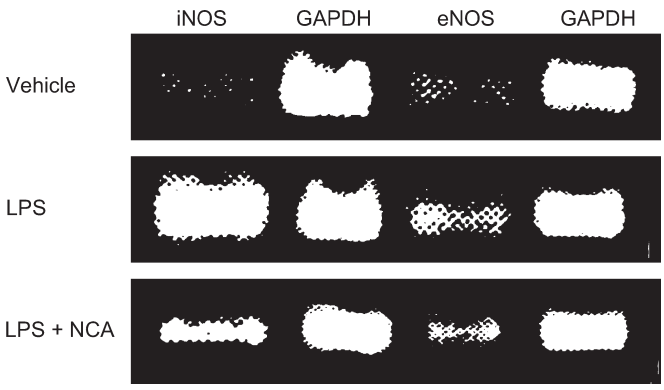

FIGURE 7. Expression of inducible and endothelial NO synthase (iNOS and eNOS) mRNA with reverse-transcriptase polymerase chain reaction in the lung tissues after various treatments. The expression of GAPDH was used as an internal standard.

In the lungs, PARS and/or PARP play key roles in the microvascular platelet-endothelial cell interaction induced by endotoxin, acute lung inflammation following intratracheal administration of LPS, or induction of asthma and leukocyte recruitment in systemic endotoxemia.[31,32,35,36] These cellular interactions, tissue inflammatory changes, and associated changes in adhesion molecules are the fundamental basis for the pathogenesis of lung injury. In the present investigation, we revealed that systemic administration of LPS to anesthetized rats caused leukocytopenia. The white blood cells reduced to a minimum at 3 h following LPS. NCA posttreatment significantly attenuated the change in leukocytes (Figure 1). The LPS-induced leukocytopenia was also observed in conscious

TABLE 1. Inducible and Endothelial Nitric Oxide Synthases (iNOS and ENOS) MRNA Expression in Lung Tissue

	iNOS/GAPDH Ratio	eNOS/GAPDH Ratio
Vehicle	0.04 ± 0.02	0.16 ± 0.06
LPS	1.68 ± 0.14*	0.78 ± 0.08*
LPS + NCA	0.92 ± 0.07*†	0.43 ± 0.04*†

Values are means ± SEM (n = 10 in each group).
$*P < 0.05$ compared with the value in vehicle.
$†P < 0.05$ compared to the value in LPS group.

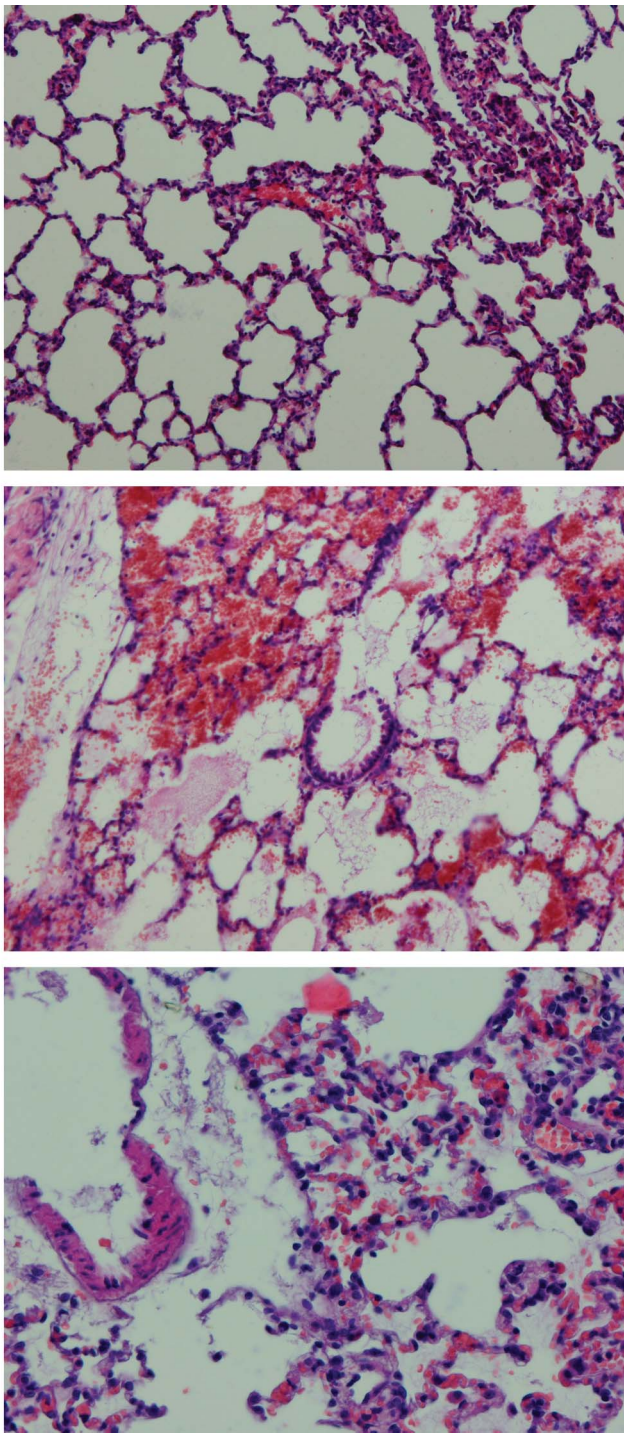

FIGURE 8. Illusive micrographs showing normal lung in anesthetized rats received vehicle (A), LPS (B), and LPS with niacinamide (C). The LPS-induced lung injury was characterized by alveolar edema and infiltration of red blood and inflammatory cells (B). Posttreatment with NCA attenuated the lung pathology (C). Hematoxylin and eosin stain, original magnification × 200.

TABLE 2. Lung Injury Score in Various Groups

	Lung Injury Score
Vehicle	0.56 ± 0.07
LPS	4.98 ± 1.34*
LPS + NCA	1.44 ± 1.03*†

Values are means ± SEM (n = 20 in each group). The data obtained in anesthetize rats and isolated lungs subjected to LPS administration with and without niacinamide (NCA) are pooled together.

*$P < 0.05$ compared with the vehicle group.

†$P < 0.05$ compared with the corresponding values in LPS group.

rats following LPS administration.[14,37] We have speculated that the leukocyte may be sequestrated into the lung from the systemic circulation. Further experimental design is required to validate this contention.

In addition to the role of PARS and PARP in the acute lung injury due to various causes, iNOS may also be a crucial factor in the lung damage. In endotoxin-induced lung damage, many studies including those from our laboratory have provided evidence to suggest that NO production through the iNOS is harmful to the lungs in various species and causes.[12,38–40] In patients with lung inflammation or injury, the iNOS expression and nitrotyrosine activity were increased.[40,41] PARP was involved in NFκB expression and thereby activated NOS. ADP-ribosylation is required to activate NFκB-mediated iNOS gene transcription.[42] Remick et al[43] also suggest that ADP ribosylation is crucial in the signaling pathway that leads to NOS mRNA expression. PARP inhibitors prevent NOS induction, iNOS mRNA expression and TNF_{α} release in interferon, and LPS-stimulated macrophages. After inhibition of PARP, iNOS expression, iNOS activity, and NO production were reduced.[32,44] PARP inhibitor also reduced the production of peroxynitrite[45] and prevented the presence of nitrotyrosine in the tissue.[27] Furthermore, nicotinamide at a concentration of 20 mM decreased mRNA of interferon regulatory factor-1, a transcription factor that plays a pivotal role in iNOS mRNA induction.[46] Park et al provided evidence to support that iNOS expression contributes to the deleterious effects exerted by PARP activation in cerebral ischemia.[30] Accordingly, there is little doubt that iNOS is involved in the pathogenesis of tissue injury following endotoxemia, ischemia-reperfusion, and other insults in various organs. In the present study, we used RT-PCR to demonstrate that LPS upregulated iNOS expression. The upregulation was attenuated by posttreatment with NCA.

Pulido et al[24] reported that PARP inhibition with 3-aminobenzamide (10 mg/kg) or nicotinamide (200 mg/kg) improved the impairment of vasorelaxation of pulmonary arterial rings in septic rats. On the other hand, these 2 PARP inhibitors given 90 min after endotoxin administration failed to prevent the lung edema formation. Their results are somehow contradictory to ours in the present study. We showed that posttreatment with niacinamide (200 mg/kg, IV) 30 min after LPS significantly reduced the extent of ALI evaluated by lung weight changes, microvascular permeability, protein, and Evans blue dye leakage. The discrepancy may be attributed to the higher dose of LPS (20 mg/kg) they used compared to that we used in this study (10 mg/kg).

In the present study, we found that pulmonary arterial pressure (PAP) was not affected by LPS. NCA increased PAP following LPS. The findings may imply that NCA acts as an iNOS inhibitor, and the reduction in NO release may account for the effects of NCA on the LPS-induced changes in PAP. In this regard, the pulmonary vasodilator effect of NO may be the sole benefit in endotoxemia. It is noteworthy that the increase in K_{fc} was not associated with the PAP changes.

CONCLUSIONS

Niacinamide protects the lungs from injury by endotoxin. This agent also ameliorates the systemic hypotension and biochemical changes indicating organ dysfunction after endotoxin administration. The mechanisms are possibly mediated through the inhibitory effects of this agent on the PARP activity and iNOS expression. Subsequently, the production of NO, free radical, and proinflammatory cytokines was suppressed, while the ATP content was restored.

ACKNOWLEDGMENTS

We are grateful to Ms. Lucy Chen (from New York University) and Ms. A. Huang for the manuscript preparation.

REFERENCES

1. Liaudet L, Szabo A, Soriano FG, et al. Poly (ADP-ribose) synthetase mediates intestinal mucosal barrier dysfunction after mesenteric ischemia. *Shock*. 2000;14:134–141.
2. Szabo C, Billiar TR. Novel roles of nitric oxide in hemorrhagic shock. *Shock*. 1999;12:1–9.
3. Ducrocq S, Benjelloun N, Plotkine M, et al. Poly(ADP-ribose) synthase inhibition reduces ischemic injury and inflammation in neonatal rat brain. *J Neurochem*. 2000;74:2504–2511.
4. Endres M, Scott G, Namura S, et al. Role of peroxynitrite and neuronal nitric oxide synthase in the activation of poly(ADP-ribose) synthetase in a murine model of cerebral ischemia-reperfusion. *Neurosci Lett*. 1998; 248:41–44.
5. Thiemermann C, Bowes J, Myint FP, et al. Inhibition of the activity of poly(ADP ribose) synthetase reduces ischemia-reperfusion injury in the heart and skeletal muscle. *Proc Natl Acad Sci USA*. 1997;94: 679–683.
6. Kiefmann R, Heckel K, Doerger M, et al. Role of PARP on iNOS pathway during endotoxin-induced acute lung injury. *Intensive Care Med*. 2004;30: 1421–1431.
7. Bowes J, Piper J, Thiemermann C. Inhibitors of the activity of poly (ADP-ribose) synthetase reduce the cell death caused by hydrogen peroxide in human cardiac myoblasts. *Br J Pharmacol*. 1998;124:1760–1766.
8. Gale EA. Molecular mechanisms of beta-cell destruction in IDDM: the role of nicotinamide. *Horm Res*. 1996;45:39–43.
9. Burkart V, Blaeser K, Kolb H. Potent beta-cell protection in vitro by an isoquinolinone-derived PARP inhibitor. *Horm Metab Res*. 1999;31: 641–644.
10. Weitberg AB. Effect of nicotinic acid supplementation in vivo on oxygen radical-induced genetic damage in human lymphocytes. *Mutat Res*. 1989; 216:197–201.
11. Chen CF, Wang D, Hwang CP, et al. The protective effect of niacinamide on ischemia-reperfusion-induced liver injury. *J Biomed Sci*. 2001;8: 446–452.
12. Lee RP, Wang D, Kao SJ, et al. The lung is the major site that produces nitric oxide to induce acute pulmonary oedema in endotoxin shock. *Clin Exp Pharmacol Physiol*. 2001;28:315–320.
13. Kao SJ, Wang D, Lin HI, et al. N-acetylcysteine abrogates acute lung injury induced by endotoxin. *Clin Exp Pharmacol Physiol*. 2006;33: 33–40.
14. Chen HI, Yeh DY, Liou HL, et al. Insulin attenuates endotoxin-induced acute lung injury in conscious rats. *Crit Care Med*. 2006;34: 758–764.
15. Hsu BG, Lee RP, Yang FL, et al. Post-treatment with N-acetylcysteine ameliorates endotoxin shock-induced organ damage in conscious rats. *Life Sci*. 2006;79:2010–2016.
16. Su CF, Yang FL, Chen HI. Inhibition of inducible nitric oxide synthase attenuates acute endotoxin-induced lung injury in rats. *Clin Exp Pharmacol Physiol*. 2007;34:339–346.
17. Kao SJ, Peng TC, Lee RP, et al. Nitric oxide mediates lung injury induced by ischemia-reperfusion in rats. *J Biomed Sci*. 2003;10:58–64.
18. Kao SJ, Wang D, Yeh DY, et al. Static inflation attenuates ischemia/reperfusion injury in an isolated rat lung in situ. *Chest*. 2004;126: 552–558.
19. Thurston G, Rudge JS, Ioffe E, et al. Angiopoietin-1 protects the adult vasculature against plasma leakage. *Nat Med*. 2000;6:460–463.
20. Muscara MN, de Nucci G. Simultaneous determination of nitrite and nitrate anions in plasma, urine and cell culture supernatants by high-performance liquid chromatography with post-column reactions. *J Chromatogr B Biomed Appl*. 1996;686:157–164.
21. Lee RP, Wang D, Lin NT, et al. Physiological and chemical indicators for early and late stages of sepsis in conscious rats. *J Biomed Sci*. 2002;9: 613–621.
22. Nakamura K, Ienaga K, Yokozawa T, et al. Production of methyl guanidine from creatinine via creatol by active oxygen species: analyses of the catabolism in vitro. *Nephron*. 1991;58:42–46.
23. Jiang Q, DeTolla L, Rooijen N, et al. Febrile-range temperature modifies early systemic tumor necrosis factor alpha expression in mice challenged with bacterial endotoxin. *Infect Immun*. 1999;67:1539–1546.
24. Pulido EJ, Shames BD, Selzman CH, et al. Inhibition of PARS attenuates endotoxin-induced dysfunction of pulmonary vasorelaxation. *Am J Physiol*. 1999;277:L769–L776.
25. Toporsian M, Govindaraju K, Nagi M, et al. Downregulation of endothelial nitric oxide synthase in rat aorta after prolonged hypoxia in vivo. *Circ Res*. 2000;86:671–675.
26. Lin NT, Yang FL, Lee RP, et al. Inducible nitric oxide synthase mediates cytokine release: the time course in conscious and septic rats. *Life Sci*. 2006;78:1038–1043.
27. Cuzzocrea S, Zingarelli B, Caputi AP. Peroxynitrate-mediated DNA strand breakage activates poly(ADP-ribose) synthetase and causes cellular energy depletion in a nonseptic shock model induced by zymosan in the rat. *Shock*. 1998;9:336–340.
28. Sakakibara Y, Mitha AP, Ayoub IA, et al. Delayed treatment with nicotinamide (vitamin B3) reduces the infarct volume following focal cerebral ischemia in spontaneously hypertensive rats, diabetic and non-diabetic Fischer 344 rats. *Brain Res*. 2002;931:68–73.
29. Slominska EM, Smolenski RT, Osborne F, et al. The effect of N-methyl-2-pyridone-5-carboxamide–A nicotinamide catabolite on poly ADP-rybosylation and oxidative stress injury in endothelial cells. *Nucleosides Nucleotides Nucleic Acids*. 2005;24:259–262.
30. Park EM, Cho S, Frys K, et al. Interaction between inducible nitric oxide synthase and poly(ADP-ribose) polymerase in focal ischemic brain injury. *Stroke*. 2004;35:2896–2901.
31. Boulares AH, Zoltoski AJ, Sherif ZA, et al. Gene knockout or pharmacological inhibition of poly(ADP-ribose) polymerase-1 prevents lung inflammation in a murine model of asthma. *Am J Respir Cell Mol Biol*. 2003;28:322–329.
32. Kiefmann R, Heckel K, Schenkat S, et al. Platelet-endothelial cell interaction in pulmonary micro-circulation: the role of PARS. *Thromb Haemost*. 2004;91:761–770.
33. Virag L, Szabo C. The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. *Pharmacol Rev.* 2002;54:375–429.
34. Boulos M, Astiz ME, Barua RS, et al. Impaired mitochondrial function induced by serum from septic shock patients is attenuated by inhibition of nitric oxide synthase and poly(ADP-ribose) synthase. *Crit Care Med*. 2003;31:353–358.
35. Kiefmann R, Heckel K, Dörger M, et al. Role of poly(ADP-ribose) synthetase in pulmonary leukocyte recruitment. *Am J Physiol Lung Cell Mol Physiol*. 2003;285:L996–L1005.
36. Liaudet L, Pacher P, Mabley JG, et al. Activation of poly(ADP-Ribose) polymerase-1 is a central mechanism of lipopolysaccharide-induced

acute lung inflammation. *Am J Respir Crit Care Med*. 2002;165: 372–377.
37. Lee CC, Lin NT, Hsu YH, et al. Inducible nitric oxide synthase inhibition potentiates multiple organ dysfunction induced by endotoxin in conscious rats. *J Cardiovasc Pharmacol*. 2005;45:396–403.
38. Kristof AS, Goldberg P, Laubach V, et al. Role of inducible nitric oxide synthase in endotoxin-induced acute lung injury. *Am J Respir Crit Care Med*. 1998;158:1883–1889.
39. Razavi HM, Wang LF, Weicker S, et al. Pulmonary neutrophil infiltration in murine sepsis: role of inducible nitric oxide synthase. *Am J Respir Crit Care Med*. 2004;170:227–233.
40. Kao SJ, Yang FL, Hsu YH, et al. Mechanism of fulminant pulmonary edema caused by enterovirus 71. *Clin Infect Dis*. 2004;38:1784–1788.
41. Haddad IY, Pataki G, Hu P, et al. Quantitation of nitrotyrosine levels in lung sections of patients and animals with acute lung injury. *J Clin Invest*. 1994;94:2407–2413.
42. Hur GM, Ryu YS, Yun HY, et al. Hepatic ischemia/reperfusion in rats induces iNOS gene transcription by activation of NF-kappaB. *Biochem Biophys Res Commun*. 1999;261:917–922.
43. Remick DG, Colletti LM, Scales WA, et al. Cytokines and extrahepatic sequelae of ischemia-reperfusion injury to the liver. *Ann N Y Acad Sci*. 1994;723:271–283.
44. Pellat-Deceunynck C, Wietzerbin J, Drapier JC. Nicotinamide inhibits nitric oxide synthase mRNA induction in activated macrophages. *Biochem J*. 1994;297:53–58.
45. Cuzzocrea S, Costantino G, Zingarelli B, et al. Protective effects of poly (ADP-ribose) synthase inhibitors in zymosan-activated plasma induced paw edema. *Life Sci*. 1999;65:957–964.
46. Akabane A, Kato I, Takasawa S, et al. Nicotinamide inhibits IRF-1 mRNA induction and prevents IL-1 beta-induced nitric oxide synthase expression in pancreatic beta cells. *Biochem Biophys Res Commun*. 1995; 215:524–530.