의학박사 학위논문

The therapeutic effect and mechanism of niacin on acute lung injury in a rat model of hemorrhagic shock: Down–regulation of the reactive oxygen species—dependent nuclear factor κB pathway

백서의 출혈성 쇼크 모델에서 나이아신의 급성 폐 손상 치료 효과 및 기전 규명: 활성 산소종 의존성 nuclear factor κB 경로 억제

2016년 2월

서울대학교 대학원
의학과 응급의학전공
정 기 영

Abstract

The therapeutic effect and mechanism of niacin on acute lung injury in a rat model of hemorrhagic shock: Down–regulation of the reactive oxygen species —dependent nuclear factor κB pathway

Ki Young Jeong

Emergency Medicine

The Graduate School

Seoul National University

Introduction: The purpose of the current study was to investigate the protective effect of niacin on acute lung injury by the down-regulation of the nuclear factor κB (NF-κB) pathway in hemorrhagic shock (HS) rats.

Methods: HS was induced in male Sprague-Dawley rats by withdrawing blood to maintain a mean arterial pressure of 20 mmHg to 25 mmHg for 40 minutes. The rats were resuscitated by the reinfusion of the drawn blood, and a vehicle (HS), a low-dose of niacin (360 mg/kg, HS + LD-NA), or a high dose of niacin (1,080 mg/kg, HS + HD-NA) were administered orally. The survival of the subjects was observed for 72 hours, and a separate set of animals was killed at 6 hours after HS induction. We measured cytoplasmic phosphorylated inhibitor κB-α and inhibitor κB-α expressions, nuclear NF-κB p65 expression, NF-κB p65 DNA-binding activity, MEK partner 1 (MP1) activity, tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), IL-8, nicotinamide adenine dinucleotide (NAD+), reduced nicotinamide adenine dinucleotide phosphate, reduced glutathione, glutathione disulfide, malondialdehyde levels, and histologic damage in the lung tissue. We also measured TNF-α, IL-6 and IL-8 levels in the serum.

Results: The survival rates of the sham, HS, HS + LD-NA, and HS + HD-NA groups were 6 of 6 (100%), 0 of 9 (0%), 1 of 9 (11.1%), and 3 of 9 (33.3%), respectively. A high dose of niacin increased lung NAD+, nicotinamide adenine dinucleotide phosphate levels, and glutathione–glutathione disulfide ratios; decreased lung malondialdehyde levels; down-regulated the NF-κB pathway; suppressed TNF-α, IL-6 and IL-8 levels in the lung tissue and serum; and attenuated histologic lung damage.

Conclusions: A high dose of niacin attenuated lung inflammation, suppressed proinflammatory cytokine release, reduced histologic lung

damage, and improved survival after HS in rats. Its therapeutic benefits were associated with the down-regulation of the reactive oxygen species–dependent NF-κB pathway.

* This work is published in J Trauma Acute Care Surg. 2015 Aug;79(2):247-55.

Keywords: hemorrhagic shock, niacin, antioxidants, acute lung injury

Student Number: 2013-30569

The List of Tables

The List of Figures

Contents

I. Introduction

During the initial phases of hemorrhagic shock (HS) following trauma, the amount of blood loss and duration of ischemia have been the main determinant of the development of multiple organ failure.(1 − 3) The current guidelines recommend adequate volume resuscitation for the recovery from ischemia induced by HS.(4, 5) However, volume resuscitation can lead to secondary damage known as an ischemia/reperfusion (I/R) injury in patients with previous tissue hypoxia due to HS. Several studies have demonstrated that I/R injury induces cellular changes via reactive oxygen species (ROS) formation, inflammatory reaction, and necrotic and apoptotic cell death.(5) Ultimately, these processes of I/R injury have been identified as the culprits in the pathogeneses of multiple-organ injuries that occur later as a result of HS.(4 − 6)

In HS patients, the lung is the most frequently involved organ, and acute lung injury (ALI) is a major cause of mortality.(7, 8) As occurs with HS, excessive formation of ROS by neutrophil infiltration following I/R injury contributes to the production of proinflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), and interleukin 8 (IL-8), which consequentially lead to the development of ALI.(9, 10) During these processes, the ROS−dependent nuclear factor κB (NF-κB) pathway plays a key role in modulating the transcription of these cytokine genes.(11, 12) In resting conditions, NF-κB dimers are sequestered in the cytoplasm by binding to inhibitor κB-α (IκB-α) in an inactive form. However, ROS induces the

phosphorylation and degradation of IκB-α, allowing the nuclear translocation of NF-κB, which results in the transcriptional activation of proinflammatory cytokine genes.(13, 14)

Niacin, a precursor of nicotinamide adenine dinucleotide (NAD+), has been widely used as a lipid-regulating medication to reduce atherosclerosis.(15) Moreover, recent researches have shed new light on its anti-inflammatory and antioxidative properties.(16 − 18) Based on its metabolic processes, niacin acts as an increasing source of reduced nicotinamide adenine dinucleotide phosphate (NADPH). Sequentially, increased levels of NADPH increase the generation of reduced glutathione (GSH) which is known as the most important intracellular antioxidant.(17, 18) During oxidative stress induced by I/R injury, GSH is oxidized to glutathione disulfide (GSSG), while ROS is eliminated via glutathione peroxidase activity, which is mainly dependent on GSH bioavailability.(19 − 21) As a result, niacin enhances this antioxidant efficacy of GSH, reduction of ROS formation, in response to oxidative stress.

Recently, several studies reported that niacin activates the down-regulation of the ROS−dependent NF-κB pathway, which reduces the release of TNF-α and IL-6 in the lungs.(18, 22 − 24) Similarly, because ALI after HS is also known to be induced by the NF-κB pathway, which results from the formation of the ROS following I/R injury, we hypothesized that niacin would also alleviate it. We are unaware of any studies evaluating the protective effects of niacin on ALI after HS. In the present study, we examined whether niacin

alleviates lung inflammation after HS in rats by down-regulating the ROS−dependent NF-κB pathway.

II. Materials and Methods

2.1 Ethics Statement

All of the experimental procedures were approved by the Animal Experiment Committee of the Institutional Animal Care and Use Committee of Seoul National University Hospital (IACUC number: 12−0357) and were conducted according to the Guidelines for Animal Experimentation from Korea Animal Protection Laws.

2.2 Animals and Drug

This experiment was conducted on 55 male Sprague-Dawley rats (320 − 390 g) purchased from the Orient Bio Inc. (Seongnam, Korea). Before experiments were performed, the rats were housed in an accredited laboratory animal center and acclimatized for at least 14 days. Food and water were provided *ad libitum*. Niacin was purchased from the Sigma-Aldrich (St. Louis, MO).

2.3 Experimental Procedures and HS Induction

Anesthetization of rats was performed as described previously.(24) The rats were anesthetized by intramuscular zoletil (zolazepam and tiletamine, Virbac AH, Fort Worth, TX) with 50 mg/kg. After anesthesia induction, each rat was intubated and mechanically ventilated using an Inspira Advanced Safety Single Animal Pressure/Volume Controlled Ventilator (Harvard Apparatus, Holliston, MA) with 2% isoflurane. Initially, volume−controlled mode (a tidal volume of 10 ml/kg and a frequency of 45 minute−1) was applied and these parameters

were manipulated to maintain the acceptable range of PCO_2 (35 − 45 mmHg). The electrocardiographic rhythm was continuously monitored. The left femoral artery and tail vein were cannulated with intravascular catheters (24 gauge, Jelco, Smiths Medical, Dublin, OH). A three-way stopcock was connected to the arterial catheter to monitor the mean arterial pressure (MAP) and blood aspiration.

HS was induced by drawing blood using a 10-ml syringe that was connected to the arterial three-way stock. Blood aspiration was performed gently and deliberately with a ratio of approximately 3 ml of blood per 100 g of body weight for 10 minutes until the MAP dropped to 20 mmHg to 25 mmHg, and the MAP was then maintained at this level for 20 minutes by further withdrawing or reinfusing drawn blood as needed. The drawn blood was stored in a heparin-coated syringe. At 30 minutes after HS induction, the vehicle, a low dose of niacin (LD-NA, 360 mg/kg), or a high dose of niacin (HD-NA, 1,080 mg/kg) diluted in distilled water to the same volume (3 ml/kg) were administered to the rats through an orogastric tube. Consecutively, the rats were resuscitated by reinfusion of the stored blood via the tail vein for 10 minutes. At 60 minutes after HS induction, the rats were weaned from the ventilator and returned to their cages.

The rats were randomly divided into four groups: (1) the rats in the sham group were administered the vehicle without HS induction; (2) the rats in the HS group were administered the vehicle after HS induction; (3) the rats in the HS + LD-NA group were administered a

low dose of niacin; and (4) the rats in the HS + HD-NA group were administered a high dose of niacin.

For survival analysis, nine rats per three intervention group (N = 27) were observed for the occurrence of mortality over 72 hours. To reveal the statistically significant mortality results, a minimum of nine rats per intervention group were required for the proper experiment sample size. When the survival rates of the HS and HS + HD-NA groups were less than 1% and 33%, respectively, which were extrapolated from pilot data, a sample size of at least nine per group was calculated as having an 80% power to detect differences at the 5% significance level.

Then, to assess the effect of niacin on lung inflammation, a separate set of animals was randomly divided into the four groups based on the survival data: the Sham group (n = 6), the HS group (n = 8), the HS + LD-NA group (n = 7), and the HS + HD-NA group (n = 7). Then, six animals in each group were killed at 6 hours after HS induction. Blood samples were obtained by cardiac puncture and centrifuged at 3,000 rpm for 10 minutes at 4°C. The separated sera were stored at −80°C for subsequent analyses. The right upper lobe of the lung in each rat was removed and fixed in 4 % formaldehyde in 0.1-M phosphate buffer. The remaining lung tissue was washed in cold physiologic saline, frozen immediately in liquid nitrogen, and kept at −80°C for biochemical analyses.

2.4 Experimental Analyses and Measurements

2.4.1 Western Blot Analysis

We performed Western blotting to determine the lung expressions of cytoplasmic phosphorylated IκB-α (p-IκB-α), IκB-α, and nuclear NF-κB p65 as previously described.(22) Briefly, lung tissue samples suspended in cold lysis buffer were homogenized at the highest setting for 2 minutes and centrifuged at 10,000 G for 10 minutes at 4 °C. The total supernatant protein concentrations were determined using the bicinchoninic acid protein assay kit (Pierce Biotechnology, Rockford, IL). Equivalent amounts of nuclear or cytoplasmic extracts (10 μg per lane) were loaded on 8 % or 12 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis, respectively. Then, the proteins were transferred onto polyvinylidene difluoride membranes (Schleicher &Schuell, Dassel, Germany). The primary antibodies used in this experiment were rabbit antirat-p-IκB-α (1:250), IκB-α (1:500), and NF-κB p65 (1:500) antibodies (Cell Signaling, Beverly, CA). The secondary antibody used was an antirabbit IgG coupled with peroxidase diluted at 1:2,000 (Stressgen, Victoria, BC, Canada). Protein bands were detected using an ECL enhanced chemiluminescence system (Amersham International, Buckinghamshire, UK) and their densities were quantified by computer-assisted densitometry of the exposed films (Lap Work Software; Seoulin Bioscience, Seoul, Korea). In addition, Western blotting for lung MEK partner 1 (MP1) expression was performed according to the manufacturer's protocol with primary anti-MP1 monoclonal antibodies (R&D system, Minneapolis, MN). All of the blots were normalized to beta actin.

2.4.2 Lung NF-κB p65 DNA-Binding Activity

Nuclear extracts from the lung tissue were prepared, and NF-κB p65 DNA-binding activities were appraised by the trans-AM method with a NF-κB p65 transcription factor assay kit (Active Motif, Carlsbad, CA).(22, 25)

2.4.3 TNF-α and IL-6 Messenger RNA Expression in the Lung by Real-Time Reverse Transcription–Polymerase Chain Reaction

TNF-α and IL-6 messenger RNA (mRNA) expressions in the lung tissue were quantified by real-time reverse transcription–polymerase chain reaction (RT-PCR) using an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA) as previously described.(22) Briefly, total RNA was extracted from frozen lung tissue using an RNeasy total RNA extraction kit (Qiagen, Chatsworth, CA). Reverse transcription to complementary DNA was performed with 2μg of RNA. The amplicons obtained in all cases were from 85 to 108 base pairs. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) was amplified for use as an endogenous control. The primers and probes used for the amplification of each gene were obtained from Applied Biosystems: TNF-α (Assav ID Rn99999017), IL-6 (Rn99999011), and GADPH (Rn99999916). Each sample was amplified in triplicate. The comparative threshold cycle method was used for the calculation of relative expression.

2.4.4 Enzyme-Linked Immunosorbent Assays

TNF-α and IL-6 levels in the serum were measured by enzyme-linked immunosorbent assays (ELISA) (R&D System, Minneapolis, MN).(18) The plates were read on a Versa Max microplate reader (Molecular

Devices Corp, Sunnyvale, CA) and measured at a wavelength of 450 nm. In addition, serum levels of IL-8 were analyzed with ELISA kit according to the manufacturer's protocol (MyBioSource, San Diego, CA). All levels in the serum were calculated according to the standard curve.

2.4.5 The Measurement of NAD+, NADPH, GSH and GSSG Levels in the Lung Tissue

NAD+ and NADPH levels in the lung tissue were measured using an NAD+/NADH Quantification Kit (BioVision, Mountain View, CA) and NADP+/NADPH quantification kits (BioVision), respectively.(18, 22) GSH and GSSG levels in the lung tissue were measured using a glutathione assay kit (Cayman Chem, Ann Arbor, MI).(18, 22)

2.4.6 The Measurement of Myeloperoxidase Activity in the Lung Tissue

Myeloperoxidase (MPO) activity was determined using an MPO colorimetric assay kit according to the manufacturer's protocol (BioVision, Milpitas, CA).(26, 27) Briefly, 50 μg of lung tissues was homogenized in 4 volumes of phosphate-buffered saline having 0.1% NP40 and centrifuged to remove insoluble materials. Supernatants were collected and mixed with MPO assay buffer and MPO substrate. The reaction mixture was incubated at room temperature for 1 hour. After stopping the reaction with Stop Mix, TNB reagent/standard was added. The absorbance was determined at 412 nm using a spectrophotometer.

2.4.7 The Measurement of Malondialdehyde Levels in the Lung Tissue

Malondialdehyde (MDA) levels were determined according to the Ohkawa method based on the reaction of thiobarbituric acid with the MDA.(22)

2.4.8 Microscopic Histology

The specimens of the right upper lung were embedded in paraffin and sectioned into 4-μm pieces. The sectioned tissue blocks were deparaffinized and stained with hematoxylin and eosin, and reviewed by a pathologist blinded to the groups. The morphologic alteration in the lungs at 6 hours after HS induction were examined by light microscopy and were assessed by a calculation of the ALI scores as previously described.(22, 25, 28) Briefly, the ALI scores contained the following four categories: (1) alveolar congestion; (2) hemorrhage; (3) infiltration or aggregation of neutrophils in air spaces or vessel walls; and (4) thickness of the alveolar wall/hyaline membrane formation. Each category was scored from 0 to 4, where a higher number is worse, giving a range of total scores from 0 to 16.

2.5 Statistical Analysis

The survival rate was estimated by the Kaplan-Meier method and compared by the log-rank test. The data are presented as the median (quartile, range). The data were analyzed by the Kruskal-Wallis test followed by the Mann-Whitney U post hoc test with Bonferroni correction. Adjusted p values of < 0.05 were considered statistically significant. All statistical analyses were conducted using SPSS for Windows version 20.0 (SPSS, Chicago, IL).

III. Results

3.1 Hemodynamic and Laboratory Data of Animals during Hemorrhagic Shock Period

Before HS induction, there were no statistically significant differences regarding the hemodynamic and biochemical variables between the groups (Table 1). During the period of HS, the MAPs in the three intervention groups were maintained within the target range (20 − 25 mmHg) (Fig. 1A).

3.2 Effect of Niacin on Survival Rate

All of the rats in the HS group were dead within 36 hours after HS induction. However, 1 (11.1%) of 9 rats in the HS + LD-NA group and 3 (33.3%) of 9 rats in the HS + HD-NA group remained alive for 72 hours after HS induction (p = 0.037) (Fig. 1B).

Table 1. Hemodynamic and Laboratory Data

	Sham (N=6)	HS (N=17)	HS+LD-NA (N=16)	HS+HD-NA (N=16)	*p* value*
Body weight, g	354.5 (340.0-370.0)	349.0 (324.0-390.0)	361.5 (331.0-386.0)	356.5 (320.0-365.0)	0.990
Drawn blood volume, mL		11.5 (10.0-12.0)	10.3 (9.4-11.5)	10.7 (10.0-12.3)	0.480
Baseline (before HS induction)					
Hemodynamics					
MAP, mmHg	85.0 (84.0-92.0)	88.0 (84.0-93.0)	84.0 (80.0-95.0)	89.5 (75.0-98.5)	0.997
Heart rate, beats/min	200.0 (190.0-240.0)	220.0 (183.0-230.0)	190.0 (188.0-231.0)	200.5 (188.0-235.0)	0.977
Arterial blood gas analysis					
pH	7.37 (7.33-7.41)	7.39 (7.34-7.41)	7.39 (7.34-7.43)	7.38 (7.35-7.41)	0.914
PCO_2, mmHg	47.3 (36.1-55.7)	45.8 (40.9-53.7)	45.8 (40.1-54.2)	44.3 (37.4-51.3)	0.889
PO_2, mmHg	153.2 (111.5-178.9)	148.7 (106.3-200.5)	154.6 (111.8-190.5)	138.9 (117.3-171.9)	0.908
HCO_3^-, mmol/L	27.8 (22.8-35.4)	29.7 (25.4-31.8)	28.5 (25.6-31.1)	27.3 (22.9-29.6)	0.500
Hemoglobin, g/dL	12.3 (11.7-13.0)	11.8 (11.0-12.8)	12.2 (11.3-12.8)	11.6 (11.1-12.3)	0.585
Lactic acid, mmol/L	2.0 (1.0-2.6)	1.9 (1.2-2.9)	1.2 (1.0-1.6)	1.8 (1.2-2.5)	0.110
30 min after HS induction					
Hemodynamics					
MAP, mmHg	96.0 (84.0-104.0)	23.0 (21.0-24.0)	22.5 (21.0-25.0)	24.0 (21.0-24.5)	0.001
Heart rate, beats/min	170.0 (158.0-209.0)	150.0 (130.0-170.0)	146.0 (136.0-181.5)	143.0 (130.0-171.0)	0.320
Arterial blood gas analysis					
pH	7.37 (7.36-7.38)	7.24 (7.16-7.26)	7.22 (7.16-7.24)	7.23 (7.16-7.27)	0.002
PCO_2, mmHg	43.9 (41.0-50.3)	43.6 (38.8-53.2)	45.7 (40.6-52.1)	40.2 (34.5-56.2)	0.896
PO_2, mmHg	138.9 (123.1-145.8)	112.4 (95.2-125.9)	118.6 (106.7-126.2)	125.1 (94.7-139.3)	0.320

HCO_3^-, mmol/L	27.9 (22.1-29.4)	18.4 (16.6-20.3)	18.4 (15.4-19.6)	17.3 (14.5-20.7)	0.040
Hemoglobin, g/dL	11.1 (10.3-11.5)	9.0 (8.0-9.3)	8.5 (7.4-9.4)	8.6 (7.9-9.2)	0.001
Lactic acid, mmol/L	1.6 (1.4-2.1)	8.0 (6.7-8.7)	7.8 (6.9-9.2)	7.7 (5.5-9.2)	0.001
60 min after HS induction					
Hemodynamics					
MAP, mmHg	99.5 (85.0-114.0)	95.0 (86.0-102.0)	89.0 (78.0-98.5)	90.5 (82.0-110.0)	0.657
Heart rate, beats/min	175.0 (150.0-180.0)	160.0 (140.0-173.0)	151.5 (136.0-174.5)	141.5 (137.5-165.0)	0.320
Arterial blood gas analysis					
pH	7.42 (7.39-7.47)	7.20 (7.14-7.28)	7.14 (7.07-7.24)	7.24 (7.12-7.27)	0.001
PCO_2,mmHg	40.5 (31.8-45.8)	56.6 (50.8-60.2)	59.6 (52.8-76.7)	51.0 (42.9-66.2)	0.018
PO_2,mmHg	142.4 (89.8-177.6)	113.7 (102.3-134.2)	115.5 (91.3-126.5)	108.0 (91.4-128.8)	0.543
HCO_3^-,mmol/L	25.7 (22.2-28.0)	22.0 (19.3-26.2)	22.3 (18.2-24.5)	22.2 (19.2-24.3)	0.226
Hemoglobin, g/dL	11.0 (10.2-11.8)	12.3 (11.7-13.2)	12.5 (11.8-12.9)	12.6 (11.5-13.4)	0.054
Lactic acid, mmol/L	1.2 (1.1-1.5)	4.2 (3.8-5.2)	3.6 (3.2-5.5)	3.3 (2.4-6.0)	0.001

*P value was calculated using Kruskal-Wallis test. Data were presented as the median (interquartile range).

HS, hemorrhagic shock; LD-NA, low dose of niacin, 360 mg/kg; HD-NA, high dose of niacin, 1,080 mg/kg; MAP, mean arterial pressure.

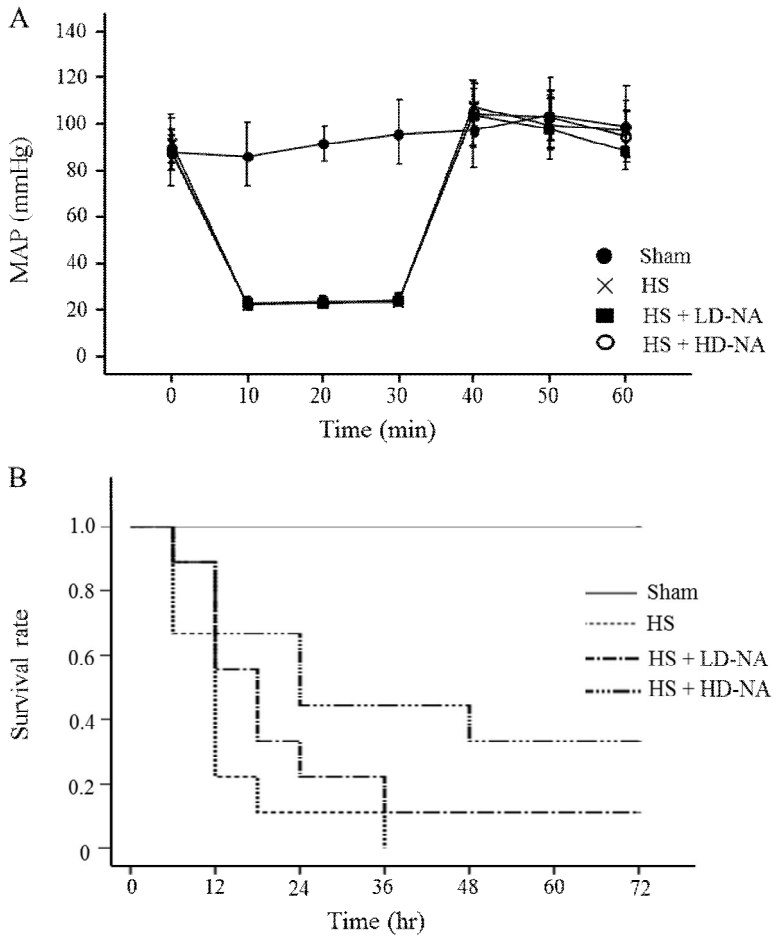

Figure 1. Time course of MAP (A) and survival for 72 hours (B) after HS induction. The Kaplan-Meier survival curve (B) shows that the survival rate in the HS + HD-NA group was significantly higher than that in the HS group (p = 0.037). MAP, mean arterial pressure; HS, hemorrhagic shock; LD-NA, low dose of niacin, 360 mg/kg; HD-NA, high dose of niacin, 1,080 mg/kg.

3.3 Effect of Niacin on the Down-Regulation of the NF-κB Pathway in the Lungs

Lung cytoplasmic p-IκB-α/IκB-α ratio, nuclear NF-κB p65 expression, and NF-κB p65 DNA binding activity in the HS + HD-NA group were significantly lower than those in the HS group (p = 0.012, 0.012, and 0.012, respectively) (Fig. 2A − D). Lung cytoplasmic p-IκB-α expression in the HS + HD-NA group was significantly lower than that in the HS group (p = 0.012) (Fig. 2A and 3A). Although not statistically significant, lung cytoplasmic IκB-α expression in the HS + HD-NA group tended to be higher than that in the HS group (p = 0.054) (Fig. 2A and 3B).

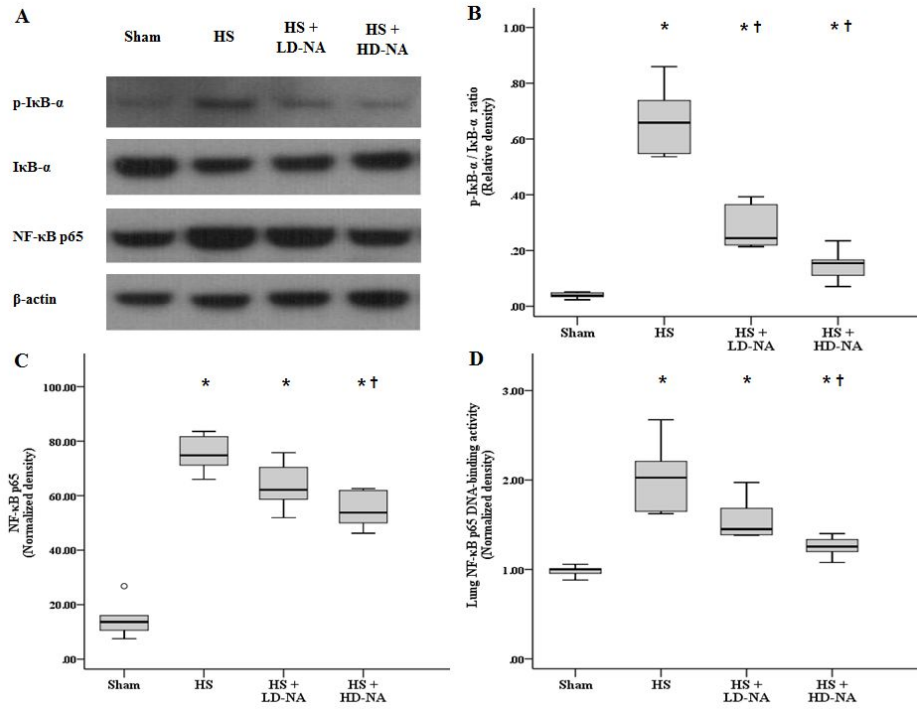

Figure 2. The effect of niacin on the NF-κB pathway. Blots were representative of the results of six rats per group (A). The cytoplasmic p-IκB-α/IκB-α ratios (B), nuclear NF-κB p65 expression (C), and NF-κB p65 DNA-binding activity (D) in the lung tissue were measured at 6 hours after HS induction. The data are presented as the median (quartile, range).

* Adjusted $p < 0.05$ versus the sham group.

† Adjusted $p < 0.05$ versus the HS group.

HS, hemorrhagic shock; LD-NA, low dose of niacin, 360 mg/kg; HD-NA, high dose of niacin, 1,080 mg/kg; IκB-α, inhibitor κB-α; p-IκB-α, phosphorylated inhibitor κB-α; NF-κB, nuclear factor κB.

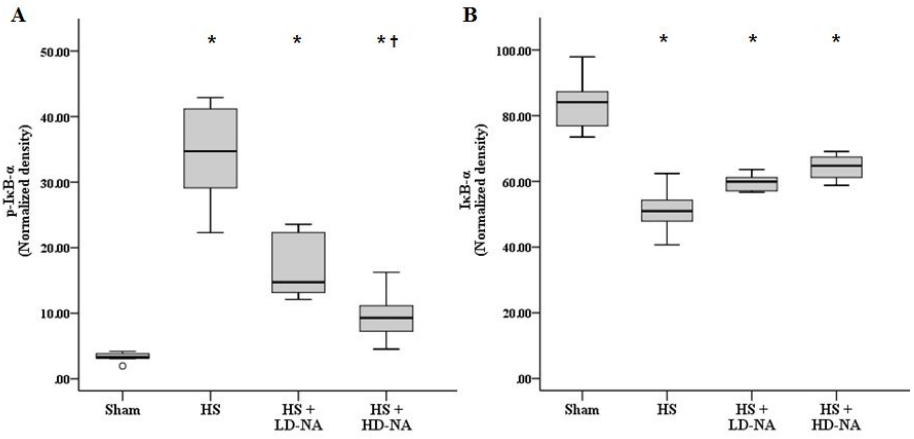

Figure 3. The cytoplasmic p-IκB-α (A) and IκB-α (B) expression in the lung tissue. The data are presented as the median (quartile, range).

* Adjusted $p < 0.05$ versus the sham group.

† Adjusted $p < 0.05$ versus the HS group.

HS, hemorrhagic shock; LD-NA, low dose of niacin, 360 mg/kg; HD-NA, high dose of niacin, 1,080 mg/kg; IκB-α, inhibitor κB-α; p-IκB-α, phosphorylated inhibitor κB-α; NF-κB, nuclear factor κB.

3.4 Effect of Niacin on the MP1, TNF-α, and IL-6 Levels in the Lung Tissue

Lung MP1 expression in the HS + HD-NA group tended to be lower than that in the HS group (p = 0.054) (Fig. 4A). Lung TNF-α and IL-6 mRNA expression in the HS + HD-NA group were lower than those in the HS group (p = 0.012 and 0.012, respectively) (Fig. 4B and C).

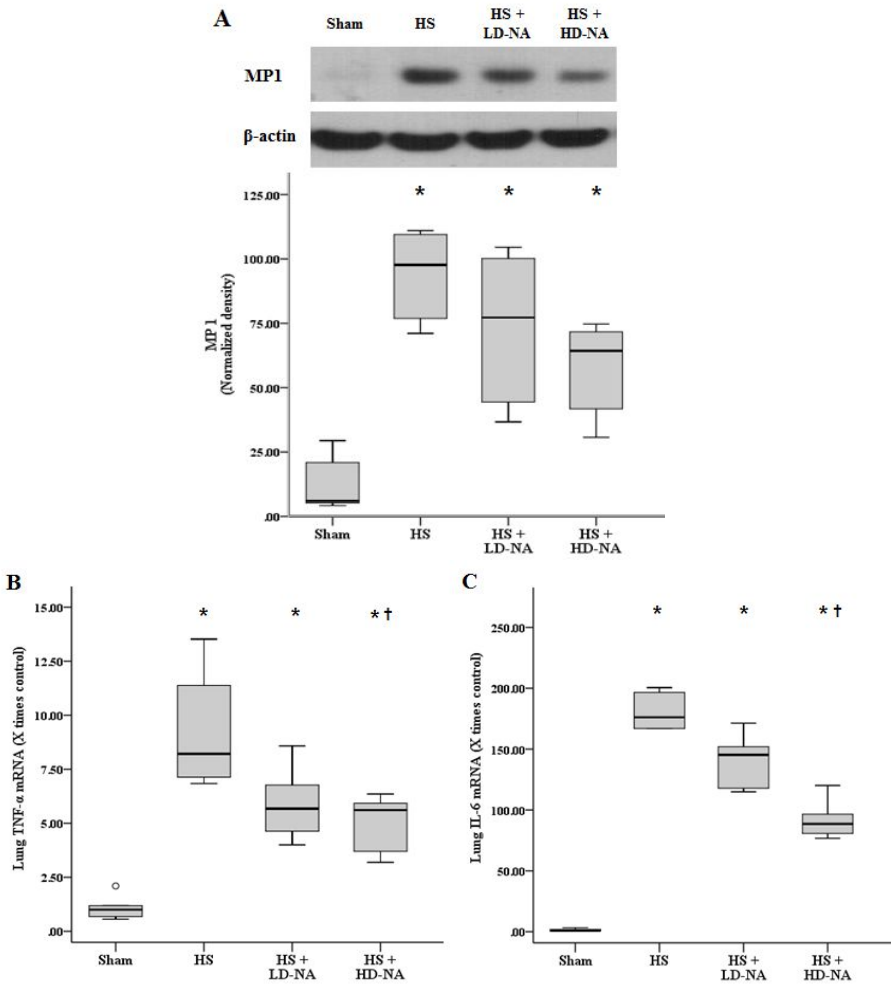

Figure 4. The effect of niacin on the production of proinflammatory cytokines in the lung tissue. Western blot analysis (six rats per group) was performed to determine the effect of niacin on lung MP1 expression (A). Lung mRNA expressions of TNF-α (B) and IL-6 (C) were determined using a real-time reverse transcription-polymerase chain reaction (RT-PCR). The data are presented as the median (quartile, range).

* Adjusted $p < 0.05$ versus. the sham group.

† Adjusted $p < 0.05$ versus. the HS group.

HS, hemorrhagic shock; LD-NA, low dose of niacin, 360 mg/kg; HD-NA, high dose of niacin, 1,080 mg/kg; MP1, MEK partner 1; TNF-α, tumor necrosis factor α; IL-6, interleukin 6.

3.5 Effect of Niacin on the TNF-α, IL-6, and IL-8 Levels in the Serum

In addition, serum TNF-α, IL-6, and IL-8 levels in the HS + HD-NA group were significantly lower than those in the HS group (p = 0.012, 0.012, and 0.012 respectively) (Fig. 5A − C).

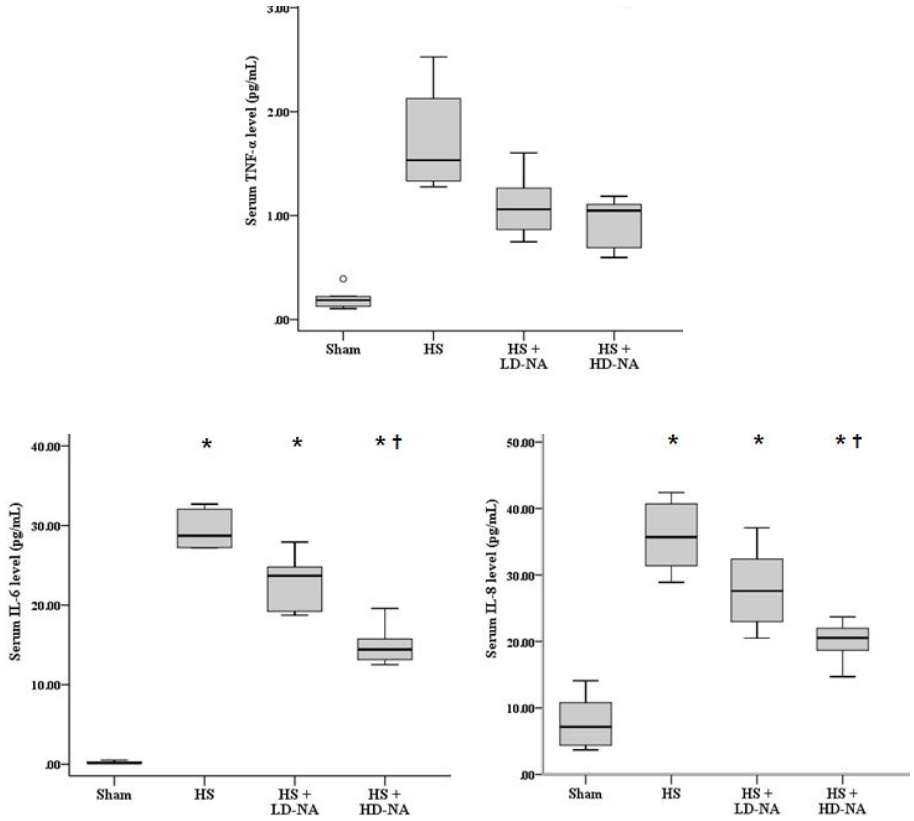

Figure 5. The effect of niacin on the production of proinflammatory cytokines in the serum. Serum levels of TNF-α (A), IL-6 (B) and IL-8 (C) were measured by enzyme-linked immunosorbent assay. The data are presented as the median (quartile, range).

* Adjusted $p < 0.05$ versus the sham group.

† Adjusted $p < 0.05$ versus the HS group.

HS, hemorrhagic shock; LD-NA, low dose of niacin, 360 mg/kg; HD-NA, high dose of niacin, 1,080 mg/kg; TNF-α, tumor necrosis factor α; IL-6, interleukin 6; IL-8, interleukin 8.

3.6 Effect of Niacin on the NAD+, NADPH, GSH, GSSG, and MDA Levels in the Lung Tissue

Lung NAD+, NADPH levels, and GSH/GSSG ratios in the HS + HD-NA group were significantly higher than those in the HS group (p = 0.024, 0.012, and 0.012, respectively) (Fig. 6A − C). Moreover, lung GSH and GSSG levels in the HS + HD-NA group were significantly higher and lower than those in the HS group, respectively (p = 0.012 and 0.012, respectively) (Fig. 6D and E). Lung MDA levels in the HS + HD-NA group were significantly lower than those in the HS group (p = 0.012) (Fig. 7).

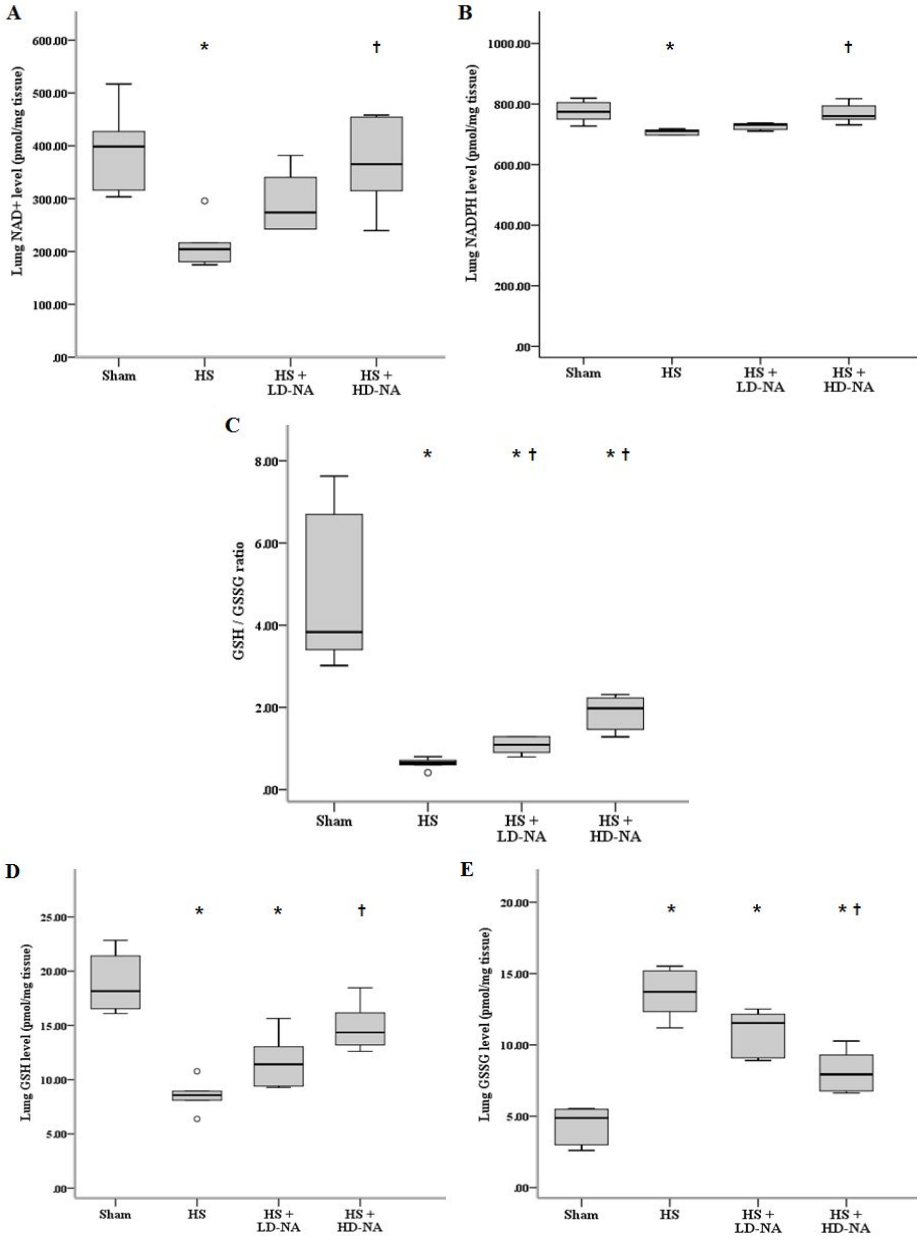

Figure 6. The antioxidant effects of niacin. Niacin increases lung NAD+ (A), NADPH (B) levels, lung GSH/GSSG ratios (C), and lung GSH levels (D), but

decreases lung GSSG levels (E). The data are presented as the median (quartile, range).

* Adjusted $p < 0.05$ versus the sham group.

† Adjusted $p < 0.05$ versus the HS group.

HS, hemorrhagic shock; LD-NA, low dose of niacin, 360 mg/kg; HD-NA, high dose of niacin, 1,080 mg/kg; NAD+, nicotinamide adenine dinucleotide; NADPH, reduced nicotinamide adenine dinucleotide phosphate; GSH, reduced glutathione; GSSG, glutathione disulfide.

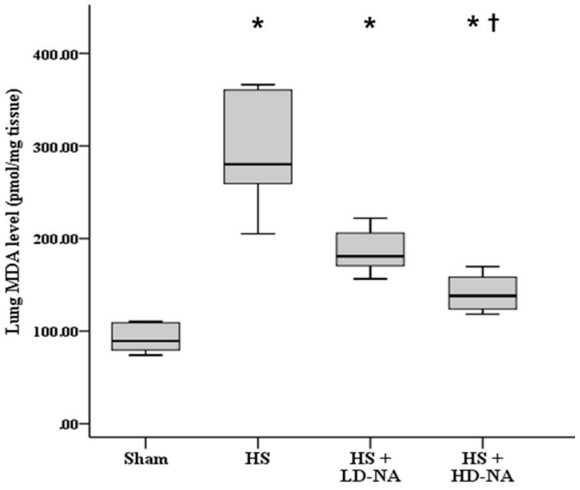

Figure 7. The antioxidant effects of niacin. Niacin decreases lung MDA levels. The data are presented as the median (quartile, range).

* Adjusted $p < 0.05$ versus the sham group.

† Adjusted $p < 0.05$ versus the HS group.

HS, hemorrhagic shock; LD-NA, low dose of niacin, 360 mg/kg; HD-NA, high dose of niacin, 1,080 mg/kg; MDA, malondialdehyde.

3.7 Effect of Niacin on Lung Histology and MPO Activity in the Lung Tissue

The protective effect of niacin, which reduces histologic damage to the lung parenchyma, is shown in figure 8. The lung MPO activities and ALI scores in the HS + HD-NA group were found to be lower than those in the HS group (p = 0.012 and 0.012, respectively) (Fig. 9A and B). The full data set of ALI scores is shown in table 2.

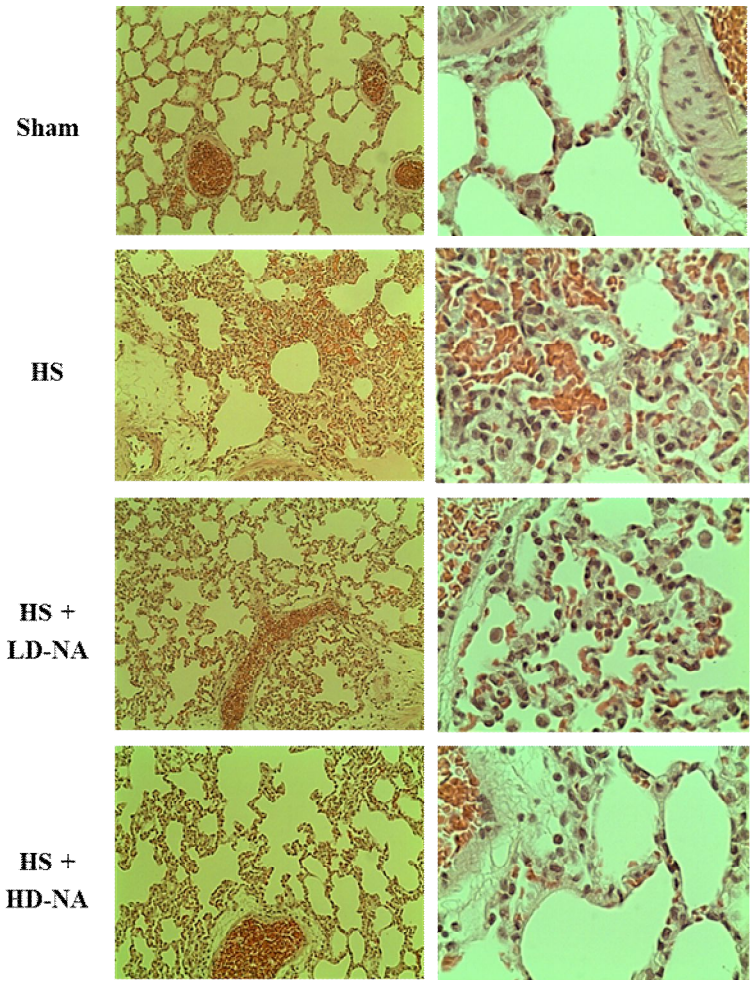

Figure 8. Microscopic histology of the lung tissue. Representative photomicrographs of lung histology (hematoxylin and eosin, x 100 and 400) show that niacin reduces lung histologic damage. The data are presented as the median (quartile, range).

* Adjusted $p < 0.05$ versus the sham group.

† Adjusted $p < 0.05$ versus the HS group.

HS, hemorrhagic shock; LD-NA, low dose of niacin, 360 mg/kg; HD-NA, high dose of niacin, 1,080 mg/kg.

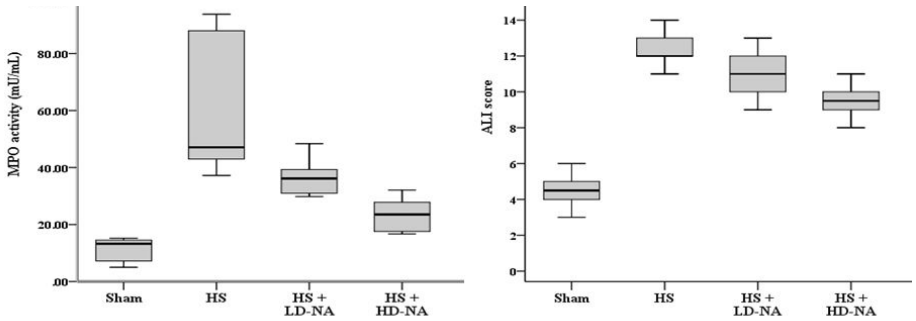

Figure 9. Lung MPO activities (A) and acute lung injury (ALI) scores (B). ALI scores show that niacin reduces lung histologic damage. The data are presented as the median (quartile, range).

* Adjusted $p < 0.05$ versus. the sham group.

† Adjusted $p < 0.05$ versus the HS group.

HS, hemorrhagic shock; LD-NA, low dose of niacin, 360 mg/kg; HD-NA, high dose of niacin, 1,080 mg/kg; MPO, myeloperoxidase; ALI, acute lung injury.

Table 2.The Full Data Set of Acute Lung Injury Scores

	Alveolar congestion	Hemorrhage	Infiltration or aggregation of neutrophils	Wall thickness / hyaline membrane formation	Sum
Sham					
#1	3	0	1	0	4
#2	2	0	1	0	3
#3	3	1	1	0	5
#4	3	0	1	0	4
#5	3	0	1	1	5
#6	2	0	2	2	6
HS					
#1	4	1	3	3	11
#2	4	3	4	3	14
#3	4	2	3	3	12
#4	4	2	3	4	13
#5	4	2	3	3	12
#6	4	1	4	3	12
HS+LD-NA					
#1	4	1	3	2	10
#2	4	0	3	2	9
#3	4	3	3	3	13
#4	4	1	3	3	11
#5	4	1	4	3	12
#6	4	1	3	3	11
HS+HD-NA					
#1	3	1	3	2	9
#2	3	1	3	3	10
#3	4	1	3	3	11
#4	4	0	2	2	8
#5	4	0	3	2	9
#6	4	1	3	2	10

The severity of lung injury was scored as follows: 0, minimum; 1, mild; 2, moderate; 3, severe; 4, maximum damage.

HS, hemorrhagic shock; LD-NA, low dose of niacin, 360 mg/kg; HD-NA, high dose of niacin, 1,080 mg/kg.

IV. Discussion

In a rat HS model, we found that HD-NA attenuates lung inflammation and improves survival. These protective effects of niacin were associated with the down-regulation of the NF-κB pathway and the suppression of proinflammatory cytokine gene expression and release. The main findings in the present study were as follows: (1) HD-NA significantly increased NAD+, NADPH levels, and GSH/GSSG ratios in the lung tissue; (2) HD-NA significantly decreased MDA levels, which are a marker for lipid peroxidation, in the lung tissue; (3) HD-NA significantly decreased lung cytoplasmic p-IκB-α/IκB-α ratios, nuclear NF-κB p65 expressions, and NF-κB p65 DNA binding activities; (4) HD-NA significantly decreased TNF-α and IL-6 mRNA expressions in the lung tissue and TNF-α, IL-6, and IL-8 levels in the serum; and (5) HD-NA significantly decreased lung MPO activities, which is an index of neutrophil accumulation in the lung tissue, and ALI scores as well as inflammations in lung histologic findings. Although significant differences between the HS group and the HS + LD-NA group were not observed for these measured data, niacin showed a dose–dependent protective effect on the development of ALI after HS. The findings in this study are consistent with our previous report showing that niacin reduces lung injury during sepsis in rats.(22)

Our data showed that the survival benefits of HD-NA were evident from 6 hours after HS induction and significant benefits were observed at 12 hours after HS induction. Previous studies also showed that

niacin plasma level peaked at 2 hours to 6 hours after its oral administration.(29, 30) Therefore, to assess the therapeutic effects of niacin, we performed blood samples and lung histologic examination at 6 hours after HS induction. In this study, unlike lung histologic findings, TNF-α, and IL-6 levels measured at 6 hours after HS induction, there were no differences in arterial gas analysis measured at 30 minutes and 60 minutes after HS induction. We considered the reason for these discordant findings may be originated from the time difference in obtaining samples from the lung tissue, cardiac puncture, and femoral artery after oral administration of niacin following HS induction. Moreover, considering the time to reach a peak level of niacin through oral routes, 2 hours to 6 hours, it also seemed to influence these discordant findings.(29, 30) However, in this study, we performed arterial gas analysis for the assessment of lung injury before the effect of niacin, not for the main outcome. This limited evaluation of lung injury before and after oral administration of niacin warrants further study using an experimental protocol in which periodic arterial gas analysis is applied during the entire process.

To build a clinically relevant model, we adjusted the total shock period to 40 minutes, 30 minutes of the induction and maintenance period and 10 minutes of the resuscitation period. In clinical settings, most patients have realistically received volume resuscitation within the first 30 minutes after HS.(31, 32) Furthermore, in the present study, the MAP was allowed to run low (20 − 25 mmHg) during the shock period. We performed this study to evaluate the therapeutic effects of

niacin on the survival rate as well as the histologic changes in the lungs after HS. Among many published studies on rat HS models,(33 − 39) the models with high MAP (≥ 35 mmHg) failed to show severe shock-related injury or mortality,(33 − 37) whereas a MAP of 20 mmHg or lower led to an expeditious and high mortality.(39) Although Rönn et al. conducted a preliminary study that resembled our experimental setting (MAP of 21 − 24 mmHg for 60 minutes), they failed to obtain consistent survival results.(39) From these data, we designed our experimental protocol of a target MAP of 20 mmHg to 25 mmHg and a total shock period of 40 minutes, and we found that this protocol was sufficient to ensure survival and organ injury. Although our animal HS model embraces feasible aspects concerning the severity, comparability, and clinical reality of HS, additional studies for an HS model with a prevailing and diverse target MAP or shock period seem to be necessary to further clarify the effect of niacin on survival. In addition, limited resuscitation and intensive care to rats with shock recovery during 72 hours after HS induction in this study may also have an influence on the mortality of those as well as the effect of severity and duration of HS on mortality. Nevertheless, it is noteworthy that there was more measurable improvement of survival in the HS + HD-NA group than in the HS group. Before clinical applications, further investigation is necessary to ascertain whether niacin attenuates ALI following HS in animal models reflecting clinical practice of HS that are commonly encountered in intensive care settings.

In the present study, we failed to measure ROS levels directly because of the use of frozen lung tissue. Instead, the MDA level, which is a marker of lipid peroxidation, was used as an indicator of oxidative stress.(40, 41) Further studies measuring the ROS levels in pulmonary microvascular endothelial cell or living tissues are warranted to confirm our data.

In the present study, the experimental dose of niacin in rats was calculated from the pharmacological dose of the drug in humans, which was 2 g/d to 4 g/d, using the body surface area normalization method.(15, 42, 43) On the basis of this converting calculation method and data in our previous experiment, we selected 360 mg/kg and 1,080 mg/kg of niacin for the low and high dose, respectively.(22, 24) Although the LD-NA used in this study corresponds well with the pharmacologic dose in humans, the correspondence between the HD-NA used in this study and the pharmacologic dose in humans does not yet permit any firm conclusions.(43, 44) Dose–related side effects of niacin, such as flushing and hepatotoxicity, have not been precisely elucidated.(45) Moreover, we used orally administered niacin in this study because of a lack of an intravenous formula; thus, the enteral absorption of niacin might be influenced by the depression of the gut absorptive capacity induced by HS.(46, 47) Accordingly, further studies regarding the safety and feasibility of niacin are warranted before the commencement of clinical trials.

V. Conclusion

In conclusion, a high dose of niacin attenuated lung inflammation, suppressed proinflammatory cytokine release, reduced histologic lung damage, and improved survival in a rat HS model. Furthermore, these therapeutic benefits seem to be associated with the down-regulation of the NF-κB pathway.

References

1. Carden DL, Granger DN. Pathophysiology of ischaemia-reperfusion injury. J Pathol. 2000;190(3):255-66.

2. Rainer TH, Lam PK, Wong EM, Cocks RA. Derivation of a prediction rule for post-traumatic acute lung injury. Resuscitation. 1999; 42(3):187-96.

3. Kauvar DS, Lefering R, Wade CE. Impact of hemorrhage on trauma outcome: an overview of epidemiology, clinical presentations, and therapeutic considerations. J Trauma. 2006;60(6 Suppl):S3-S11.

4. Rizoli SB, Rhind SG, Shek PN, Inaba K, Filips D, Tien H, et al. The immunomodulatory effects of hypertonic saline resuscitation in patients sustaining traumatic hemorrhagic shock: a randomized, controlled, double-blinded trial. Ann Surg. 2006;243(1):47-57.

5. Gruen RL, Brohi K, Schreiber M, Balogh ZJ, Pitt V, Narayan M, et al. Haemorrhage control in severely injured patients. Lancet. 2012; 380(9847):1099-108.

6. McGhan LJ, Jaroszewski DE. The role of toll-like receptor-4 in the development of multi-organ failure following traumatic haemorrhagic shock and resuscitation. Injury. 2012;43(2):129-36.

7. Davidson GH, Hamlat CA, Rivara FP, Koepsell TD, Jurkovich GJ, Arbabi S. Long-term survival of adult trauma patients. JAMA. 2011; 305(10):1001-7.

8. Ware LB, Matthay MA. The acute respiratory distress syndrome. N Engl J Med. 2000;342(18):1334-49.

9. Shenkar R, Abraham E. Mechanisms of lung neutrophil activation after hemorrhage or endotoxemia: roles of reactive oxygen intermediates, NF-kappa B, and cyclic AMP response element binding protein. J Immunol. 1999;163(2):954-62.

10. Eltzschig HK, Eckle T. Ischemia and reperfusion-from mechanism to translation. Nat Med. 2011;17(11):1391-401.

11. Shenkar R, Yum HK, Arcaroli J, Kupfner J, Abraham E. Interactions between CBP, NF-kappaB, and CREB in the lungs after hemorrhage and endotoxemia. Am J Physiol Lung Cell Mol Physiol. 2001;281(2):L418-L426.

12. Pantano C, Reynaert NL, van der Vliet A, Janssen-Heininger YM. Redox-sensitive kinases of the nuclear factor-kappaB signaling pathway. Antioxid Redox Signal. 2006;8(9-10):1791-806.

13. Morgan MJ, Liu ZG. Crosstalk of reactive oxygen species and NF-κB signaling. Cell Res. 2011;21(1):103-15.

14. Fan J, Ye RD, Malik AB. Transcriptional mechanisms of acute lung injury. Am J Physiol Lung Cell Mol Physiol. 2001;281(5): L1037-L1050.

15. Meyers CD, Kamanna VS, Kashyap ML. Niacin therapy in atherosclerosis. Curr Opin Lipidol. 2004;15(6):659-65.

16. Kamanna VS, Kashyap ML. Nicotinic acid (niacin) receptor agonists: will they be useful therapeutic agents? Am J Cardiol. 2007; 100(11A):S53-S61.

17. Yan Q, Briehl M, Crowley CL, Payne CM, Bernstein H, Bernstein C. The NAD+ precursors, nicotinic acid and nicotinamide upregulate

glyceraldehyde-3-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase mRNA in Jurkat cells. Biochem Biophys Res Commun. 1999;255(1):133-6.

18. Ganji SH, Qin S, Zhang L, Kamanna VS, Kashyap ML. Niacin inhibits vascular oxidative stress, redox-sensitive genes, and monocyte adhesion to human aortic endothelial cells. Atherosclerosis. 2009; 202(1):68-75.

19. Gloire G, Piette J. Redox regulation of nuclear post-translational modifications during NF-kappaB activation. Antioxid Redox Signal. 2009;11(9):2209-22.

20. Ji LL, Fu RG, Waldrop TG, Liu KJ, Swartz HM. Myocardial response to regional ischemia and reperfusion in vivo in rat heart. Can J Physiol Pharmacol. 1993;71(10-11):811-17.

21. Jaeschke H. Reactive oxygen and ischemia/reperfusion injury of the liver. Chem Biol Interact. 1991;79(2):115-36.

22. Kwon WY, Suh GJ, Kim KS, Kwak YH. Niacin attenuates lung inflammation and improves survival during sepsis by downregulating the nuclear factor-κB pathway. Crit Care Med. 2011;39(2):328-34.

23. Gurujeyalakshmi G, Wang Y, Giri SN. Taurine and niacin block lung injury and fibrosis by down-regulating bleomycin-induced activation of transcription nuclear factor-kappaB in mice. J Pharmacol Exp Ther. 2000;293(1):82-90.

24. Kwon WY, Suh GJ, Kim KS, Lee HJ, Jeong KY, Kwak YH, et al. Niacin suppresses the mitogen-activated protein kinase pathway and

attenuates brain injury after cardiac arrest in rats. Crit Care Med. 2013;41(9):e223-e232.

25. Kwon WY, Suh GJ, Kim KS, Jo YH, Lee JH, Kim K, et al. Glutamine attenuates acute lung injury by inhibition of high mobility group box protein-1 expression during sepsis. Br J Nutr. 2010; 103(6):890-8.

26. Mullane KM, Kraemer R, Smith B. Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischemic myocardium. J Pharmacol Methods. 1985;14(3):157-67.

27. Masuda Y, Vaziri ND, Takasu C, Li S, Robles L, Pham C, et al. Salutary effect of pre-treatment with an Nrf2 inducer on ischemia reperfusion injury in the rat liver. Gastroenterol Hepatol (Que). 2014; 1(1):1-7.

28. Nishina K, Mikawa K, Takao Y, Maekawa N, Shiga M, Obara H. ONO-5046, an elastase inhibitor, attenuates endotoxin-induced acute lung injury in rabbits. Anesth Analg. 1997;84(5):1097-103.

29. Lauring B, Rosko K, Luo WL, Wenning L, Kissling J, Roupe K, et al. A comparison of the pharmacokinetics of two different formulations of extended-release niacin. Curr Med Res Opin. 2009;25(1):15-22.

30. Lundholm L, Jacobsson L, Brattsand R, Magnusson O. Influence of nicotinic acid, niceritrol and beta-pyridylcarbinol on experimental hyperlipidemia and atherosclerosis in mini-pigs. Atherosclerosis. 1978; 29(2):217-39.

31. Bulger EM, May S, Kerby JD, Emerson S, Stiell IG, Schreiber MA, et al. and the ROC investigators. Out-of-hospital Hypertonic

Resuscitation After Traumatic Hypovolemic Shock: A Randomized, Placebo Controlled Trial. Ann Surg. 2011;253(3): 431-41.

32. Rahbar E, Fox EE, del Junco DJ, Harvin JA, Holcomb JB, Wade CE, et al. and the PROMMTT Study Group. Early resuscitation intensity as a surrogate for bleeding severity and early mortality in the PROMMTT study. J Trauma Acute Care Surg. 2013;75(1 Suppl 1): S16-S23.

33. Hoppen RA, Corso CO, Grezzana TJ, Severino A, Dal-Pizzol F, Ritter C. Hypertonic saline and hemorrhagic shock: hepatocellular function and integrity after six hours of treatment. Acta Cir Bras. 2005; 20(6):414-17.

34. Lee C, Xu DZ, Feketeova E, Nemeth Z, Kannan KB, Haskó G, et al. Calcium entry inhibition during resuscitation from shock attenuates inflammatory lung injury. Shock. 2008;30(1):29-35.

35. Mathis KW, Sulzer J, Molina PE. Systemic administration of a centrally acting acetylcholinesterase inhibitor improves outcome from hemorrhagic shock during acute alcohol intoxication. Shock. 2010; 34(2):162-8.

36. Molina MF, Whitaker A, Molina PE, McDonough KH. Alcohol does not modulate the augmented acetylcholine-induced vasodilatory response in hemorrhaged rodents. Shock. 2009;32(6):601-7.

37. Legrand M, Mik EG, Balestra GM, Lutter R, Pirracchio R, Payen D, et al. Fluid resuscitation does not improve renal oxygenation during hemorrhagic shock in rats. Anesthesiology. 2010;112(1):119-27.

38. Zhong Z, Enomoto N, Connor HD, Moss N, Mason RP, Thurman RG. Glycine improves survival after hemorrhagic shock in the rat. Shock. 1999;12(1):54-62.

39. Rönn T, Lendemans S, de Groot H, Petrat F. A new model of severe hemorrhagic shock in rats. Comp Med. 2011;61(5):419-26.

40. Pawar A, Nanduri J, Yuan G, Khan SA, Wang N, Kumar GK, et al. Reactive oxygen species-dependent endothelin signaling is required for augmented hypoxic sensory response of the neonatal carotid body by intermittent hypoxia. Am J Physiol Regul Integr Comp Physiol. 2009;296(3):R735-R742.

41. de Cavanagh EM, Inserra F, Ferder L, Fraga CG. Enalapril and captopril enhance glutathione-dependent antioxidant defenses in mouse tissues. Am J Physiol Regul Integr Comp Physiol. 2000;278(3): R572-R577.

42. Desai VG, Lee T, Moland CL, Branham WS, Von Tungeln LS, Beland FA, et al. Effect of short-term exposure to zidovudine (AZT) on the expression of mitochondria-related genes in skeletal muscle of neonatal mice. Mitochondrion. 2009;9(1):9-16.

43. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J. 2008;22(3):659-61.

44. van der Hoorn JW, de Haan W, Berbée JF, Havekes LM, Jukema JW, Rensen PC, et al. Niacin increases HDL by reducing hepatic expression and plasma levels of cholesteryl ester transfer protein in APOE*3Leiden.CETP mice. Arterioscler Thromb Vasc Biol. 2008; 28(11):2016-222.

45. Goldberg AC. Clinical trial experience with extended-release niacin (Niaspan): dose－escalation study. Am J Cardiol. 1998;82(12A):35U-41U.
46. Singh G, Chaudry KI, Chudler LC, Chaudry IH. Depressed gut absorptive capacity early after trauma-hemorrhagic shock. Restoration with diltiazem treatment. Ann Surg. 1991;214(6):712-18.
47. Chang JX, Chen S, Ma LP, Jiang LY, Chen JW, Chang RM, et al. Functional and morphological changes of the gut barrier during the restitution process after hemorrhagic shock. World J Gastroenterol. 2005;11(35):5485-91.

Author contributions

Conception and study design: Ki Young Jeong, Gil Joon Suh, and Woon Yong Kwon

Acquisition of data: Ki Young Jeong, Kyung Su Kim, Yoon Sun Jung, and Yu Chan Kye

Data analysis and interpretation: Woon Yong Kwon and Kyung Su Kim

Draft and critical revision: Ki Young Jeong, Gil Joon Suh, and Woon Yong Kwon

Acknowledgements

This research was supported by a Research Program funded by National Medical Center, Research Institute (grant number: NMC 2012－MS－10). All authors have made substantive contributions to the study, and all authors endorse the data and conclusions. The authors are grateful to Sung Hee Kim for technical assistance and Kyung Bun Lee for the histologic review.

요약(국문초록)

백서의 출혈성 쇼크 모델에서 나이아신의 급성 폐 손상 치료 효과 및 기전 규명: 활성 산소종 의존성 nuclear factor κB 경로 억제

정 기 영

의학과 응급의학전공

서울대학교 대학원

서론: 출혈성 쇼크(hemorrhagic shock, HS)에서 나이아신의 투여가 nuclear factor κB (NF-κB)의 경로를 억제함으로써 급성 폐 손상을 줄이고 생존을 향상시키는지를 알아보고자 이번 연구를 시행하였다.

방법: 출혈성 쇼크는 스프라그-다울리(Sprague-Dawley) 종의 수컷 백서를 이용하여 마취 후 대퇴부 절개를 통해 대퇴동맥에서 채혈을 함으로써 유발하였고, 20-25 mmHg의 평균동맥압으로 40분 동안 유지하였다. 출혈성 쇼크 유발 후, 소생을 위하여 채혈된 혈액을 다시 주입하면서 동시에 기초제(vehicle, HS군), 저용량 나이아신(360 mg/kg, HS + LD-NA군), 고용량 나이아신(1,080 mg/kg, HS + HD-NA군)을 투여하였다. 생존여부 확인을 위해 출혈성 쇼크 유발 이후 72시간 동안 백서를 관찰하였고 장기 손상여부 확인을 위해 출혈성

쇼크 유발 이후 6시간째 채혈과 조직 적출을 시행하였다. 각 군에 대해 혈역학적 변화 측정, 동맥혈 검사를 시행하였고, 적출된 폐 조직에서 cytoplasmic phosphorylated inhibitor κB-α와 inhibitor κB-α의 발현, nuclear NF-κB p65의 발현, NF-κB p65 DNA 결합 활성도, MEK partner 1 (MP1)의 활성도, tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), IL-8, nicotinamide adenine dinucleotide (NAD+), reduced nicotinamide adenine dinucleotide phosphate (NADPH), reduced glutathione (GSH), glutathione disulfide (GSSG), malondialdehyde, 조직학적 손상 정도를 측정하였다. 또한 혈액에서 TNF-α, IL-6, IL-8을 측정하였다.

결과: Sham군, HS군, HS + LD-NA군, HS + HD-NA군에서의 생존율은 각각 100% (6/6), 0% (0/9), 11.1% (1/9), 33.3% (3/9)이었다. 고용량 나이아신의 투여는 폐 NAD+, NADPH, GSH/GSSG 비를 유의하게 증가시켰고 폐 malondialdehyde를 유의하게 감소시켰다. 또한 고용량 나이아신은 NF-κB 경로를 억제하여 폐 조직과 혈액내의 TNF-α, IL-6, IL-8를 유의하게 감소시켰으며, 병리학적 소견을 통한 폐 손상도 감소시키는 소견을 보였다.

결론: 백서의 출혈성 쇼크 모델에서 고용량의 나이아신 투여는 폐 염증을 호전시키고 전염증성 사이토카인 (proinflammatory cytokine) 방출을 줄이며 병리학적 폐 손상을 줄여 최종적으로 생존율을 증가시켰다. 이러한 나이아신의 치료 효과는 활성산소종 의존성 NF-κB 경로의 억제와 연관이 있었다.

* 본 내용은 J Trauma Acute Care Surg. 2015 Aug;79(2):247-55에 출판 완료된 내용임

주요어: 출혈성 쇼크, 나이아신, 항산화제, 급성 폐 손상
학 번: 2013-30569