

Niacin attenuates acute lung injury induced by lipopolysaccharide in the hamster

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Niacin attenuates acute lung injury induced by lipopolysaccharide in the hamster. A. Nagai, S. Yasui, Y. Ozawa, H. Uno, K. Konno. ©ERS Journals Ltd 1994.

ABSTRACT: Lipopolysaccharide plays a major role in the development of lung injury induced by Gram-negative bacteria, but a protective agent to attenuate the LPS-induced lung injury has not been found. The aim of this study was to examine the effects of niacin on LPS-induced acute lung injury.

We administered LPS (*Escherichia coli*) 0.01 mg·100 g⁻¹ body weight, transtracheally into the lungs of hamsters. Niacin (250 or 500 mg·kg⁻¹ body weight) was injected intraperitoneally 24 h before, and 1 h after the LPS administration.

LPS treatment increased wet/dry lung weight, albumin content and neutrophil counts in bronchoalveolar lavage fluid. In hamsters treated with niacin, wet/dry lung weight, albumin content and intra-alveolar cell counts were normal. Nicotinamide adenine dinucleotide (NAD) was significantly decreased in lung tissue of hamsters treated with LPS alone, but was increased in hamsters treated with LPS and niacin. Histopathological examination revealed that niacin-treated LPS-administered hamsters had lungs with no or occasional inflammatory cell infiltration in alveolar spaces, in contrast to the lungs of LPS-treated hamsters, which were infiltrated with numerous inflammatory cells.

We conclude that niacin attenuates LPS-induced acute lung injury, probably, in part, by preventing the depletion of NAD.

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Lipopolysaccharide (LPS) components of Gram-negative bacteria (endotoxin) have a high biological activity and are presumed to cause injury to the lungs [1]. In clinical settings, Gram-negative pneumonia is known to be a risk factor for the development of adult respiratory distress syndrome (ARDS), and it has been suggested that LPS plays a role in the pathogenesis of ARDS [2]. It has also been suggested that LPS is the major causative agent in the development of respiratory problems induced by organic dust inhalation [3].

Niacin has been found to prevent cellular injury [4], and also to reduce lung injury induced by paraquat [5], or bleomycin [6]. Although the mechanism of niacin protection remains unknown, one explanation for the protective effect is that deoxyribonucleic acid (DNA) damage caused by toxic agents leads to the consumption of nicotinamide adenine dinucleotide (NAD) through the synthesis of poly adenosine diphosphate (ADP)-ribose, and that the administration of niacin prevents the depletion of NAD, resulting in prevention of cellular injury [7, 8]. Endotoxin (LPS) may directly or indirectly damage DNA by means of LPS-reactive substances, such as oxygen radicals [1, 9], proteinases [1] and cyclic nucleotide [10]. Niacin has been used clinically in the management of type II hyperlipoproteinaemia or familial hypertriglyceridaemia as a lipid-lowering agent [11]. There

is no effective agent for satisfactory management of acute lung injury induced by endotoxin. Consequently, we studied the effects of niacin on LPS (*Escherichia coli*)-induced acute lung injury in hamsters.

Materials and methods

The study protocol is shown in Figure 1. Adult Syrian male golden hamsters (weighing 136±3 g) purchased from Charles River Breeding Laboratories (Kanagawa, Japan) were randomly assigned to one of five groups as follows: 1) LPS-administered and 500 mg niacin-treated group (LN₅₀₀ group) - six animals under anaesthesia were transtracheally instilled with *E. coli*-LPS (Sigma Chemical Co., St. Louis, MO, USA) 0.01 mg·per 100 g⁻¹ body weight (BW) dissolved in 0.2 ml of sterile saline solution. Niacin (500 mg·kg⁻¹ BW) dissolved in 0.5 ml of saline solution was adjusted to pH 6.3 and intraperitoneally injected 24 h before, 30 min before and 1 h after LPS injection; 2) LPS-administered and 250 mg niacin-treated group (LN₂₅₀ group) - six animals were given the same dose of LPS intratracheally as the LN₅₀₀ group and intraperitoneally injected with niacin (250 mg·kg⁻¹ BW, pH 6.3); 3) LPS-administered and saline-treated group (LS group) - eight animals received the same dose of

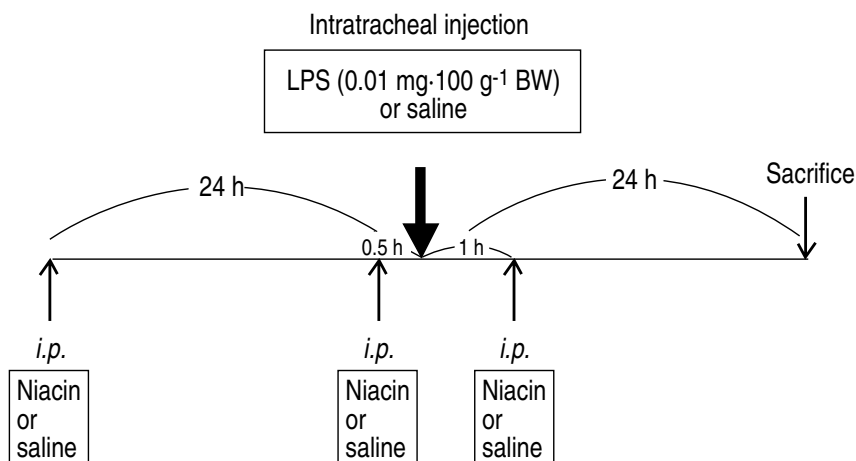


Fig. 1. – Study design. This shows the protocol of LPS-treated (with or without niacin treatment) animals and saline-injected control animals. LPS: lipopolysaccharide; *i.p.*: intraperitoneal injection.

LPS as mentioned above, administered intratracheally and were then intraperitoneally injected with the same volume of saline (pH 6.3); 4) saline-administered and niacin-treated group (SN group) - eight animals were given an intratracheal injection of 0.2 ml of saline and intraperitoneally injected with niacin 500 mg·kg⁻¹ (pH 6.3); 5) saline control group (SS group) - eight animals were given the same volume of saline (pH 6.3) as mentioned above intratracheally and intraperitoneally.

One day after LPS injection, the animals were killed with an intraperitoneal injection of sodium pentobarbital. After the animals were weighed, the chest wall was excised, the lungs were examined and the right lung was excised and weighed immediately. The dry weight of the lungs was determined by drying them in a laboratory oven for 2 days, and the wet/dry lung weight was calculated. After insertion of a tracheal tube, bronchoalveolar lavage (BAL), with 2 ml of sterile saline at room temperature, was performed three times serially in the left lung through a cannulated tube. For histopathological studies, in separate experiments (five animals in each group) the lungs were inflated with 2.5% glutaraldehyde buffered with cacodylate for at least 48 h at a constant pressure of 20 cmH₂O.

BAL fluid. The recovered BAL fluid was filtered through a single layer of gauze to remove mucus. Cells in the lavage fluid were counted with a haemocytometer, and differential counts were performed on Wright-stained preparations. The supernatant of the lavage fluid was used to measure protein and albumin contents, according to the methods of LOWRY *et al.* [12] and RODKEY [13], respectively.

Leucocyte count. Blood samples were obtained by cardiac puncture and total leucocyte counts were performed using a haemocytometer.

NAD measurement. In separate experiments, five animals in each group, except the LN₂₅₀ group, were used to measure lung NAD. After the lungs were perfused with cold isotonic saline solution, the right lungs were dis-

sected and immediately frozen in liquid nitrogen and stored at -80°C. The dry frozen tissue was weighed and finely powdered in the frozen state, and NAD was extracted with perchloric acid. The NAD content of the lung was determined by the enzymatic method of KLINGENBERG [14]. The NAD in lung tissue was expressed as µg·g⁻¹ dry lung tissue.

Endotoxin assay. To determine whether niacin directly inactivates LPS, endotoxin was evaluated in a mixture of LPS and niacin by the method of OBAYASHI *et al.* [15].

Histopathology. From the fixed lungs, transverse sections (5 µm thick) in 3 planes (medial one-third, midsagittal and two-thirds of the lung) were obtained and stained with haematoxylin and eosin. The severity of inflammatory cell infiltration to the lung was assessed using lung sections. Each lung was blindly assigned an inflammatory score as follows: 1 = rare or occasional inflammatory cells scattered through the lung; 3 = abundant inflammatory cells scattered through the lung; 2 = inflammatory cells between 1 and 3. The total inflammation score for each animal was calculated as the mean of the six lung scores (six sections from right and left lungs).

The results are expressed as the mean±SEM. The five groups of animals were compared by analysis of variance and Student-Newman-Keul's tests. Probability values of less than 0.05 were considered significant.

Results

No animals died during the experiment. There were no significant differences in the body weight: SS 137±4 g; SN 141±1 g; LS 139±3 g; LN₅₀₀ 133±4 g; and LN₂₅₀ 135±5 g.

The wet/dry lung weight is shown in figure 2. Wet/dry lung weight was significantly increased in the LS group (4.5±0.08), but the weight ratios in both L₅₀₀ (4.2±0.05) and LN₂₅₀ (4.0±0.15) groups were identical to those in the SN (4.1±0.07) and SS (4.1±0.05) groups.

The volume of BAL fluid recovered from animals did not differ among the groups. The mean recovery rate

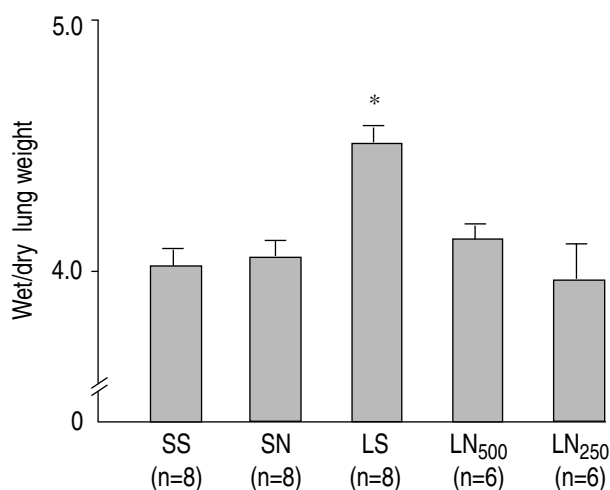


Fig. 2. – Wet/dry lung weight. SS group: saline (*i.t.*) + saline (*i.p.*); SN group: saline (*i.t.*) + niacin 500 mg·kg⁻¹ BW (*i.p.*); LS group: LPS (*i.t.*) + saline (*i.p.*); LN₅₀₀ group: LPS (*i.t.*) + niacin 500 mg·kg⁻¹ BW (*i.p.*); LN₂₅₀ group: LPS (*i.t.*) + niacin 250 mg·kg⁻¹ BW (*i.p.*). LPS: lipopolysaccharide; BW: body weight; *i.t.*: intratracheal injection; *i.p.*: intraperitoneal injection. *: significantly higher ($p < 0.05$) than all other groups. SN, LN₅₀₀ and LN₂₅₀ groups were not different from the SS group.

was 82±0.4%. Total protein content in BAL fluid was increased in the LS group (367±21 µg·ml⁻¹), but the LN₅₀₀ group (138±33 µg·ml⁻¹), LN₂₅₀ group (158±27 µg·ml⁻¹) and SN group (107±6 µg·ml⁻¹) were not different from the SS group (105±9 µg·ml⁻¹). Albumin content in BAL fluid was increased in the LS group (190±30 µg·ml⁻¹), but albumin was not detected (<20 µg·ml⁻¹) in the LN₅₀₀, LN₂₅₀, SN and SS groups. The total number and cellular population of intra-alveolar cells obtained from BAL fluid is shown in table 1. The total number of intra-alveolar cells was higher in the LS group than in the LN₅₀₀, LN₂₅₀, SN and SS groups. Analysis of the cellular population revealed that neutrophils were more than tenfold higher in the LS group than in the other groups. However, there was no difference in the total number of alveolar macrophages and lymphocytes among the five groups.

There was no difference in circulating leucocyte counts among LS (4.17±0.61 cells×10⁶·ml⁻¹), LN₅₀₀ (5.89±1.85), LN₂₅₀ (7.03±1.20), SN (6.52±0.62) and SS (8.37±1.63) groups.

Table 1. – Intra-alveolar cell number in bronchoalveolar lavage fluid

Group	n	Total cell (×10 ⁵ ·ml ⁻¹)	Macrophage (×10 ⁵ ·ml ⁻¹)	Neutrophil (×10 ⁵ ·ml ⁻¹)	Lymphocyte (×10 ⁵ ·ml ⁻¹)
SS	8	1.81±0.14	1.13±0.13	0.44±0.04	0.24±0.03
SN	8	1.40±0.30	1.35±0.77	0.01±0.02	0.04±0.03
LS	8	26.20±8.18*	2.94±1.20	23.16±7.70*	0.10±0.08
LN ₅₀₀	6	2.58±1.06	1.00±0.50	1.51±1.06	0.07±0.03
LN ₂₅₀	6	2.79±0.79	0.90±0.68	1.84±1.04	0.06±0.01

Data are presented as mean±SEM. *: significantly different ($p < 0.05$) from SS group. SS group: saline (*i.t.*) + saline (*i.p.*); SN group: saline (*i.t.*) + niacin 500 mg·kg⁻¹ BW (*i.p.*); LS group: LPS (*i.t.*) + saline (*i.p.*); LN₅₀₀ group: LPS (*i.t.*) + niacin 500 mg·kg⁻¹ BW (*i.p.*); LN₂₅₀ group: LPS (*i.t.*) + niacin 250 mg·kg⁻¹ BW (*i.p.*); LPS: lipopolysaccharide; *i.t.*: intratracheal injection; *i.p.*: intraperitoneal injection.

Table 2. – NAD concentrations in lung tissue at 24 h after transtracheal injection of LPS or saline

Group	n	NAD (µg·g ⁻¹ of lung)
SS	5	0.285±0.009
SN	5	0.308±0.015
LS	5	0.235±0.004*
LN ₅₀₀	5	0.477±0.007*

Data are represented as mean±SEM. *: significantly different ($p < 0.05$) from SS group. NAD: nicotinamide adenine dinucleotide. For further abbreviations see legend to table 1.

Table 3. – Endotoxin assay in mixture of LPS and niacin

	Endotoxin (pg·ml ⁻¹)
LPS alone (0.01 mg)	1103.8±12.1
LPS (0.01 mg) + niacin (20 µg)	1168.8±15.5
LPS (0.01 mg) + niacin (40 µg)	1116.8±49.5
LPS (0.01 mg) + niacin (400 µg)	1183.9±153.1

Values are mean of three samples. Data are presented as mean±SEM. Total volume of mixture = 0.2 ml. The concentrations of niacin were decided with reference to [15]. There were no significant differences between mixtures. LPS: lipopolysaccharide.

The NAD content of lung tissue is shown in table 2. When compared with the SS group, the LS group showed a significant decrease, the LN₅₀₀ group showed a significant increase, and the SN group had a higher mean value but the difference was not statistically significant.

Table 3 presents endotoxin measurements in the mixture of LPS and niacin. LPS was not inactivated by the addition of various concentrations of niacin.

Histological examination by light microscopy revealed that the lungs of the LN group showed a smaller number of inflammatory cells in intra-alveolar spaces, in contrast to the LS group, in which the lungs showed thickened alveolar septa, intra-alveolar haemorrhage and infiltration of numerous inflammatory cells in the intra-alveolar and/or interstitial spaces (fig. 3). These lesions in the LS group were diffusely scattered throughout both lungs. The lungs of the SS and SN groups appeared normal. The inflammation scores in the lungs are compared in table 4. The LS group had a significantly higher score, and the average scores of the LN₅₀₀ and LN₂₅₀ groups were slightly higher than those of the SN and SS groups.

Table 4. – Comparison of severity of alveolar inflammation

Group	n	Inflammation score
SS	6	1.06±0.04
SN	6	1.08±0.06
LS	6	2.61±0.17*
LN ₅₀₀	6	1.53±0.08‡
LN ₂₅₀	6	1.64±0.11‡†

*: significantly different ($p < 0.05$) from SS, SN, LN₅₀₀, and LN₂₅₀ groups. ‡: significantly different ($p < 0.05$) from SS group; †: significantly different ($p < 0.05$) from SN group. For abbreviations see legend to table 1.

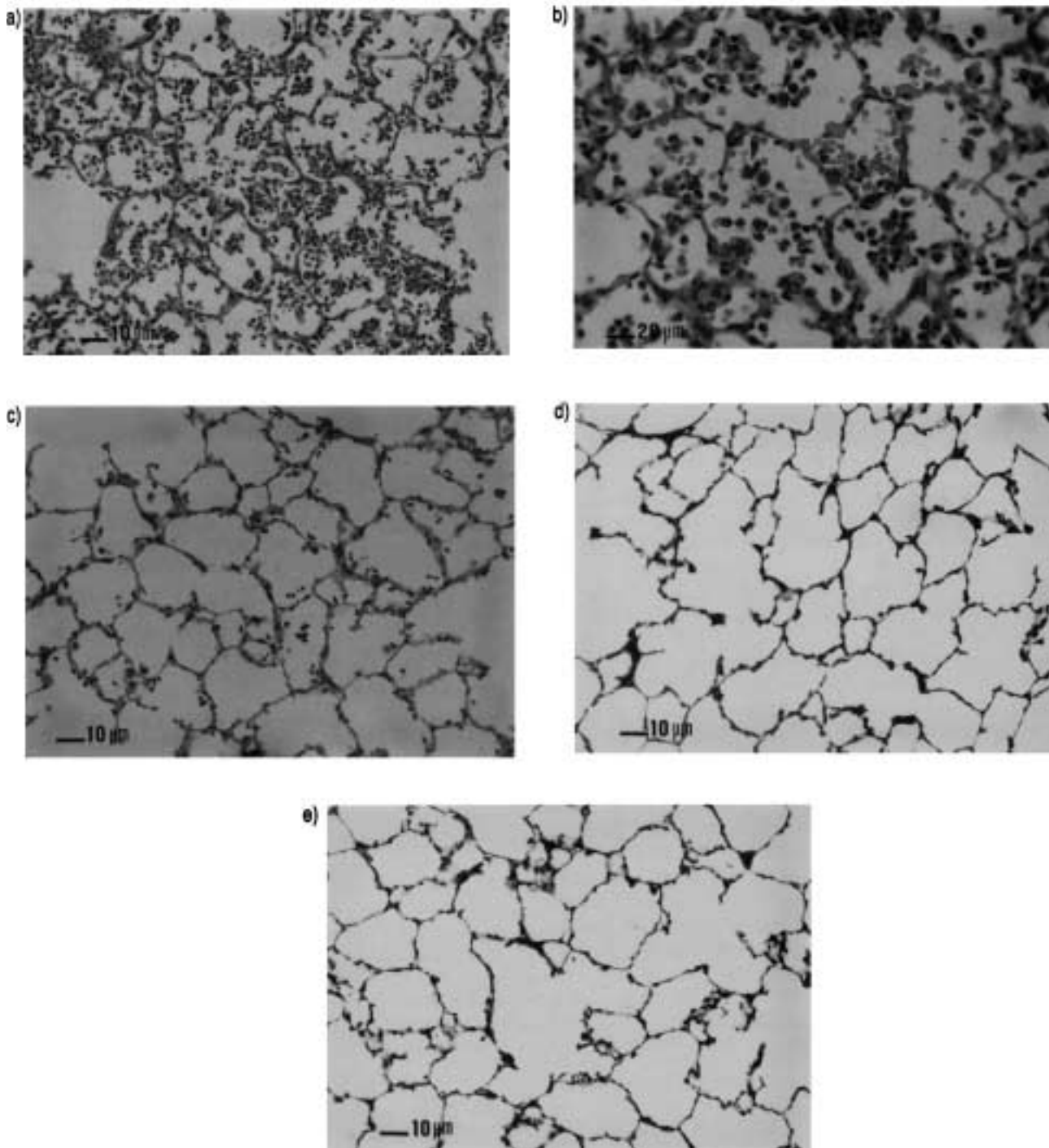


Fig. 3. – Light photomicrographs of the lungs. a) Lungs of LPS-treated animals (LS group) show abundant inflammatory cell infiltration into intra-alveolar and interstitial spaces. b) High power magnification of LS group shows numerous neutrophils in alveolar spaces. c) Animals treated with LPS and niacin $500 \text{ mg}\cdot\text{kg}^{-1} \text{ BW}$ (LN_{500} group) have lungs with a small number of inflammatory cells in the alveoli. d) Animals treated with saline and niacin $500 \text{ mg}\cdot\text{kg}^{-1} \text{ BW}$ (SN group) had lungs which were normal in appearance. e) Animals treated with saline (SS group) had lungs which were normal.

Discussion

This study clearly demonstrated that niacin attenuated LPS-induced acute lung injury, characterized by increased lung weight, increased protein and albumin content in

the alveolar fluid, and intra-alveolar or interstitial infiltration of inflammatory cells.

In this study, 0.01 mg of LPS was administered into the lungs by the transtracheal route, since a preliminary study showed that this dose of LPS produced sufficient

acute lung injury, whilst an intraperitoneal injection of LPS did not produce acute lung injury in hamsters. In addition, a previous study using hamsters suggested that inhalation of LPS is reasonable for a model of endotoxin-induced lung injury [16]. At the beginning of this study, we considered the possibility that in hamsters weighing 135 g, 0.2 ml instilled into the lungs would not be distributed throughout the alveoli, and would not produce inflammatory changes diffusely throughout the lungs. However, the lungs in the LPS-administered animals in our study had diffusely scattered inflammatory changes consisting of intra-alveolar haemorrhage and inflammatory cells. These findings are consistent with the morphological changes seen in intravenously endotoxin-infused animals [17–21]. Therefore, our animal preparation appears to be appropriate as a model of endotoxin-induced lung injury.

In attempting to determine the effect of niacin, the route of administration of LPS should be disregarded. LPS transtracheally injected into the lung would first affect macrophages and epithelial cells in intra-alveolar airspaces. In the present study, LPS-treated animals showed a greater increase in the number of intra-alveolar neutrophils, but not macrophages. LPS-treated animals also showed obvious morphological changes as described previously, but niacin-treated animals did not. These findings suggest that systematically-administered niacin possibly protects pulmonary parenchyma cells from LPS or LPS-associated inflammatory substances, such as tumour necrosis factor and interleukins, which may be released from LPS-stimulated macrophages.

Two different doses of niacin (250 and 500 mg·kg⁻¹ BW), chosen after consideration of previous studies [5, 6], strikingly attenuated LPS-induced lung injury. What was the mechanism of the effect of niacin? Niacin did not inactivate LPS directly, and had no effect on circulating leukocyte number. One possible explanation of the mechanism is that administration of niacin protects cells from the depletion of NAD, which results in cell lysis. In our study, animals treated with LPS alone showed a decrease in lung NAD, whereas animals treated with LPS and niacin had 2 and 1.5 times as much NAD as the animals treated with LPS alone and saline-control animals, respectively. Recent studies have shown that LPS from *E. coli* causes endothelial injury [22, 23]. The cellular injury is mediated by toxic metabolites of oxygen released from activate inflammatory cells [24, 25]. Hydrogen peroxide, a metabolite of oxygen, can induce cellular damage *via* activation of poly-ADP-ribose polymerase [9, 26], resulting in intracellular depletion of NAD. As shown in our study, depletion of NAD can be prevented by addition of niacin. Therefore, niacin treatment was considered to inhibit cellular damage through inhibition of depletion of NAD, resulting in prevention of acute lung injury.

In the present study, there was no dose effect of niacin. This might reflect the fact that we have chosen a very high dose of niacin and one which exceeds the range of dose effects. Concerning NAD measurements, NAD concentration in lung tissue was measured in the LN₅₀₀ group but not in the LN₂₅₀ group, since a dose effect of niacin

was largely absent. In these measurements the number of animals studied is somewhat smaller, but seems large enough since the variation of NAD measurements was very small in each group.

The precise mechanisms of acute lung injury induced by endotoxin remains unclear. LPS-induced acute lung injury is considered to result from known tissue injury factors, such as oxidants and proteases [1]. These findings may lead to the other possible mechanisms, that niacin may provide protection against such factors. However, inhibitors of protein and prostaglandin synthesis, cytoskeletal function, protease and phospholipid activity, and oxygen radical production have failed to attenuate the injury [1, 22]. Therefore, this seems not to be the reason for the niacin effects in our study. HARLAN *et al.* [22] have shown that cycloheximide, a protein synthesis inhibitor, enhances endotoxin injury, and they indicate the importance of protein synthesis for protection from endotoxin-induced cellular damage. However, BROWN *et al.* [5] have shown that niacin treatment results in body weight loss, suggesting the possibility of alterations in the metabolic state. Although body weight was not different in LPS and/or niacin-treated animals, the possibility that effects of niacin are due to alterations of protein synthesis remains.

The albumin content as well as protein in BAL fluid was measured as an index of pulmonary permeability. LPS-treated animals had higher concentrations of BAL albumin, suggesting increased permeability of the lung, and LPS-niacin-treated animals showed BAL albumin concentration identical to that of saline control animals. These findings indicate that niacin prevented the leakage of serum albumin into the airspace. However, in bleomycin-treated animals, niacin has failed to prevent increased pulmonary vascular permeability [6]. Why niacin affected LPS-induced lung injury but not bleomycin-induced injury remains uncertain. A difference in the mechanism of the injury between LPS and bleomycin may lead to different effects of niacin. Thus, lung NAD was decreased in the LPS-treated hamsters, but not in the bleomycin-treated animals. Other points are the differences in duration of niacin administration and the experimental period. In our study, niacin was administered one day before and immediately before and after the LPS injection, and in the bleomycin study niacin was injected daily for 3 weeks, from 2 days before bleomycin injection until the animals were sacrificed.

This study showed that niacin prevented the accumulation of neutrophils within the alveolar space. One mechanism to explain this was that addition of niacin reduced circulating neutrophil numbers, resulting in the absence of neutrophils in the lungs of niacin-treated animals and preventing leukocyte accumulation into the lungs. However, this seems not to be the case, since there was no difference in the number of circulating leucocytes among the experimental animals and the controls.

In this study, we included niacin-control animals (intratracheal injection of saline and intraperitoneal injection of niacin), to ascertain whether niacin has unexpected effects on the lungs. However, niacin-control animals showed histology, BAL fluid and wet/dry lung weight

identical with those of saline-control animals. Therefore, we conclude that niacin does not have any adverse effect on the lungs.

In conclusion, niacin administration prevented the LPS-induced lung injury and showed no detectable adverse effects. These experiments are likely to be comparable to the lung injury seen in pneumonia induced by Gram-negative bacteria. Although the dose of niacin used was 50–100 fold greater than that recommended for humans, niacin therapy might have an opportunity for success for treatment of these pneumonias that are often necrotizing or acute lung injury (ARDS) induced by sepsis. However, the clinical utility of niacin requires further study.

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