Multiple factors contributing to lipopolysaccharide-induced reactivity changes in rabbit pulmonary artery

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Abstract: To explore the underlying mechanism(s) of pulmonary arterial hypertension in endotoxic shock, the roles of N-acetylcysteine (NAC), nitric oxide (NO) and carbon monoxide (CO) were investigated. Pulmonary arterial rings (3-mm width) were prepared from 24 rabbits. Lipopolysaccharide (LPS), after 7-hour incubation, decreased the endothelium-dependent relaxation response of the arterial ring (pre-contracted with phenylephrine) to acetylcholine (1 µmol/L), but did not affect the endothelium-independent relaxation response to sodium nitroprusside. The LPS effects were reduced by a concomitant incubation with the free radical scavenger (NAC), NO donor (L-arginine), and CO donor (hemin), respectively. On the other hand, the LPS effects were enhanced by applying heme oxygenase-1 (HO-1) inhibitor (zinc protoporphyrin) to block CO production. The response to acetylcholine changed from relaxation to contraction, however, the contractile response to phenylephrine increased significantly after pre-incubation with nitric oxide synthase (NOS) inhibitor (L-NAME) to block NO production, confirming the importance of CO and NO. These results show that LPS impairs endothelium-dependent relaxation of the pulmonary artery, which can be greatly reduced by the antioxidant, or by supplying with NO and CO. Thus, multiple factors are involved in this model of endotoxin-induced pulmonary hypertension.

Key words: N-acetylcysteine; lipopolysaccharide; nitric oxide; carbon monoxide; pulmonary artery

Lipopolysaccharide (LPS), a main component of Gram-negative bacterial endotoxin[1], is the leading cause of endotoxic shock. The early stage of endotoxic shock is characterized by systemic hypotension and pulmonary arterial hypertension, and further, the degree and duration of pulmonary arterial hypertension may contribute to the acute...
lungs injury during endotoxic shock\textsuperscript{2}. Overproduction of endothelial nitric oxide (NO) has been identified to play an important role in the increased pulmonary artery pressure\textsuperscript{3, 4}. 

\textit{In vivo} study indicated that LPS could increase inducible nitric oxide synthase (iNOS) at mRNA and protein level\textsuperscript{3}, however, its role in pulmonary arterial hypertension has not been fully understood.

Heme oxygenase (HO) catalyzes the oxidation of heme to carbon monoxide (CO) and biliverdin, which is then converted to bilirubin by biliverdin reductase. So far three isoforms of HO have been identified: the inducible HO-1, 2 and 3\textsuperscript{6}. And study revealed that HO-1 was a NO/cGMP-sensitive endothelial gene and CO released by HO-1 conferred protection against vasoconstriction induced by hypoxia\textsuperscript{7}.

In addition, \textit{N}-acetylcysteine (NAC) treatment has been shown to ameliorate structural lung damage by reducing pulmonary vascular resistance in endotoxin-exposed rats\textsuperscript{8}, but its role in the development of pulmonary arterial hypertension induced by LPS has not been well understood.

In the present study, we hypothesize that NAC, NO and CO are all involved in the pathogenesis of pulmonary hypertension induced by endotoxin.

1 MATERIALS AND METHODS

1.1 Animals and tissues

Adult male New Zealand white rabbits (2.0–3.0 kg) were sacrificed, and the pulmonary arteries were rapidly isolated as described before\textsuperscript{9}. In brief, the pulmonary arteries were placed in 4°C modified Krebs solution containing (in mmol/L) NaCl 118, KCl 4.7, CaCl\textsubscript{2} 2.5, MgSO\textsubscript{4} 1.2, KH\textsubscript{2}PO\textsubscript{4} 1.2, NaHCO\textsubscript{3} 25, and glucose 11.1 (pH 7.2–7.4). Once cleaned of adventitia, the pulmonary artery was cut into 3-mm width rings. Care was taken during this process to avoid endothelial injury. Then pulmonary arterial rings were maintained in RPMI-1640 medium (GibcoBRL) supplemented with 100 U/ml penicillin and 100 µg/ml streptomycin and were randomly divided into four groups incubated at 37°C with different agents in a humidified atmosphere with 5% CO\textsubscript{2}. Pulmonary artery rings were divided into four groups at random: Vehile group (medium containing saline as vehicle), LPS group (medium containing 4 µg/ml LPS, LPS+NAC group (medium containing 4 µg/ml LPS and 0.5 mmol/L NAC) and NAC group (medium containing 0.5 mmol/L NAC). After being incubated for 7 h, each ring was threaded between two stainless steel parallel hooks and suspended in tissue bath (37°C) filled with 6 ml Krebs solution for the measurement of isometric tension. The top hook was connected to an isometric force-displacement transducer, and the bottom hook was anchored to an immovable support. Tissue baths were continuously bubbled with 95% O\textsubscript{2} and 5% CO\textsubscript{2}.

Artery rings from 4 to 6 rabbits were studied in each group. Rings were stretched to resting tension of 4.0 g and allowed to equilibrate for 1 h, during which time Krebs solution was changed every 15 min before experiment. The experiments were conducted after the rings were rinsed with Krebs solution and the tension returned to the baseline.

1.2 Experiment protocols

1.2.1 Demonstration of vasoreactive changes to acetylcholine and phenylephrine

After equilibration, endothelium-intact rings from all groups were challenged with 1 µmol/L α\textsubscript{1}-adrenoceptor agonist phenylephrine. At the peak of contraction, the endothelium-dependent relaxation response to 1 µmol/L acetylcholine (ACh) and the endothelium-independent relaxation response to 1 µmol/L sodium nitroprusside were tested. Four to six rabbits (8–12 rings) were studied in all groups. Rings were rinsed with Krebs solution and returned to baseline tension, followed by the following experiment.

1.2.2 Role of NO in the vasoreactive changes

To determine whether NO contributed to the decreased relaxant responsiveness induced by LPS, NO donor \textit{L}-arginine (2 mmol/L), iNOS inhibitor aminoguanidine (0.1 mmol/L) and NOS inhibitor \textit{L}-NAME (0.1 mmol/L) were added to the tissue bath respectively and then 1.2.1 was repeated 15 min after every agent added.

1.2.3 Role of CO in the vasoreactive changes

To determine the role of CO in the decreased relaxant responsiveness induced by LPS, CO donor hemin (0.02 mmol/L) and HO-1 inhibitor zinc protoporphyrin (0.01 mmol/L) were added to the tissue bath respectively and then 1.2.1 was repeated 20 min after every agent added.

1.3 Drugs

LPS (0111:B4), NAC, \textit{L}-arginine, aminoguanidine, \textit{L}-NAME, hemin, zinc protoporphyrin, sodium nitroprusside, acetylcholine and phenylephrine were purchased from Sigma. RPMI-1640 medium was purchased from GibcoBRL. All the other chemicals were purchased from Chinese Chemical Co.

1.4 Data analysis

Statistical analysis was performed with SPSS software. Data are presented as mean ± SD. Relaxation is expressed as percentage of the active tension generated by phenylephrine. Statistical significance was obtained by one-way analysis of variance (ANOVA) and Newman-Keuls \textit{q} test. \textit{P}<0.05 was accepted to be statistically significant.
2 RESULTS

2.1 Effect of NAC on pulmonary artery exposed to LPS

Pulmonary artery rings contraction in response to α₁-adrenergic receptor phenylephrine (1 µmol/L) agonist was not impaired by in vitro LPS administration. In contrast, the endothelium-dependent relaxation response of pulmonary artery rings to 1 µmol/L ACh was significantly decreased by LPS, which, however, could be reversed by a concomitant exposure to NAC. NAC alone had no affection on the pulmonary artery vasoreactivity (Fig.1, 2). The endothelium-independent relaxation response of pulmonary artery to sodium nitroprusside was unaffected by LPS (data not shown).

2.2 Effect of L-arginine, aminoguanidine, L-NAME, hemin or zinc protoporphyrin on the vasoreactivity of pulmonary artery rings

The endothelium-dependent relaxation response of pulmonary artery to acetylcholine can be totally or partially restored by L-arginine and hemin in the LPS group, and L-arginine and hemin had synergic effect. Neither the relaxation response to ACh nor the contractile response to phenylephrine was affected by aminoguanidine in each group. Pretreatment with L-NAME led to switch from the relaxation response to ACh to contractile response, and the contractile response to phenylephrine was significantly increased as well (Fig.3, 4).

In LPS group, pretreatment with zinc protoporphyrin led to significantly decreased relaxation response to ACh and increased contraction response to phenylephrine (Fig. 5, 6).

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Fig. 1. Representative tracings showing tension of relaxation to acetylcholine in isolated rabbit pulmonary artery rings. Relaxation responses of pulmonary artery rings to acetylcholine after incubation with vehicle (A), LPS (B), NAC+ LPS (C) and NAC (D) for 7 h. W, wash with Krebs solution. 1 0, 1 µmol/L phenylephrine; 11, 1 µmol/L ACh.

Fig. 2. Effect of NAC on LPS-induced decrease in the relaxation responses of pulmonary artery rings to ACh after incubation for 7 h. *P<0.05 vs vehicle, †P<0.05 vs LPS.

Fig. 3. Effect of L-Arg (L-arginine), AG (aminoguanidine) and L-NAME on endothelium-dependent relaxation responses of pulmonary artery rings to acetylcholine. n=8. *P<0.05 vs control, †P<0.01 vs AG.

Fig. 4. Effect of AG (aminoguanidine) and L-NAME on contraction responses of pulmonary artery rings to phenylephrine. n=6. *P<0.05 vs control, †P<0.01 vs AG.
DISCUSSION

Previous studies showed that LPS injection in the rat resulted in a kind of acute lung injury characterized by lung neutrophile accumulation, edema, increase of pulmonary vascular resistance, and dysfunction of vasorelaxation to cGMP-mediated agonists\(^{[10,11]}\). Although early production of NO by endothelial NOS (eNOS) after LPS administration appears to play a protective role in acute lung injury, the later phase of endotoxemia or endotoxic shock results in the induction of iNOS in pulmonary artery, with subsequent production of NO and its cytotoxic derivative peroxynitrite\(^{[12,13]}\). Increase of pulmonary iNOS activity induced by LPS has been extensively studied, however, its role in the pulmonary circulation has not been well understood. In the present study, we found that pulmonary artery with LPS exposure had decreased endothelium-dependent relaxation response to ACh, however, the endothelium-independent relaxation response to sodium nitroprusside and vasoconstriction to phenylephrine were not significantly affected. The LPS effects could be reduced by a concomitant incubation with NO donor (\(L\)-arginine). Neither the relaxation response nor the augment of contractile response was observed with aminoguanidine, the selective iNOS inhibitor in all groups. The response to ACh changed from relaxation to contraction, however, the contractile response to phenylephrine increased significantly after pre-incubation with NOS inhibitor (\(L\)-NAME) to block NO production. This finding was likely due to inhibition of eNOS, and confirmed the importance of endothelial-derived NO in maintaining a low basal tone in the pulmonary circulation. Although in our present experiment it seems that iNOS inhibitor aminoguanidine has no effect on the pulmonary artery vasomotor function during LPS exposure, it might be important to attenuate or even abrogate other aspects of acute lung injury\(^{[14]}\). Selective iNOS inhibition by either aminoguanidine or \(S\)-methylisothiourea can prevent the increase of lung iNOS activity and the reduction of lung vascular leak\(^{[15,16]}\) in animal models of LPS-induced acute lung injury. In addition, iNOS knockout mice has no significant changes in lung NOS activity, lactate dehydrogenase activity, lung wet/dry ratio, or pulmonary nitrotyrosine staining after LPS injection, indicating that iNOS deficiency mice in gene are more resistant to LPS-induced acute lung injury than wild-type mice\(^{[17]}\).

These data suggest that the decreased relaxation response of pulmonary artery contributes to the development of pulmonary artery hypertension in endotoxic shock, and the reduced production of NO by eNOS might be critical. The discovery of endogenous gaseous messenger, CO, has been moving the research of pulmonary arterial hypertension induced by LPS to a very new phase. But the effect and significance of HO/CO on the development of pulmonary artery hypertension have not been fully understood. In this study, we observed that the decreased endothelium-dependent relaxation of pulmonary artery induced by LPS can be partially reversed by CO donor, and hemin and \(L\)-arginine showed synergistic effect. On the other hand, the LPS effects could be enhanced by HO-1 inhibitor (zinc protoporphyrin) through blocking the CO production. Our findings suggested an important protective function of HO-1/CO was as a vasorelaxant to supply the place of NO by eNOS whose function has been impaired by LPS in endotoxic shock. In addition, the free radical scavenger (NAC) could also reduce the LPS effects.

In summary, these results show that LPS impairs endothelium-dependent relaxation of the pulmonary artery, which can be greatly reduced by the antioxidant, or by supplying with NO and CO. Thus, multiple factors are involved in this model of endotoxin-induced pulmonary hypertension.
REFERENCES


