Lipopolysaccharide-Induced Vascular Endothelial Growth Factor Expression in Rat Lung Pericytes

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저 자 씀

CONTENTS

Abstract ··· 1

Ⅰ. INTRODUCTION ··· 3

Ⅱ. MATERIALS AND METHODS ··· 5

1. Primary culture of rat lung pericytes ··· 5
2. Experimental design ··· 8
3. RNA extraction and reverse-transcription polymerase chain reaction ··· 8
4. Enzyme-linked immunosorbent assay ··· 9
5. Western blot analysis ··· 10
6. Statistical analysis ··· 11

Ⅲ. RESULTS ··· 12

1. Expression of VEGF mRNA in pericytes stimulated with LPS ··· 12
2. Effects of LPS on VEGF protein expression in cultured pericytes ··· 14
3. Role of iNOS on LPS-induced VEGF mRNA expression ··· 16
4. Effect of p38 MAP kinase on LPS-induced VEGF mRNA expression ··· 19

Ⅳ. DISCUSSION ··· 21

Ⅴ. CONCLUSION ··· 23

REFERENCES ··· 25

Abstract (in Korean) ··· 29

LIST OF FIGURES

Figure 1. Photomicrographs of rat lung pericytes in culture ························ 7

Figure 2. Time and dose response of LPS-induced VEGF mRNA expression in cultured rat lung pericytes ···································· 13

Figure 3. Enhanced VEGF protein expression in rat lung pericytes stimulated with LPS ··· 15

Figure 4. Effects of LPS on VEGF and iNOS mRNA level in cultured rat lung pericytes ··· 17

Figure 5. Effects of pharmacological interventions on the LPS induced expression of VEGF mRNA in cultured rat lung pericytes ····· 18

Figure 6. Involvement of p38 MAP kinase in enhanced VEGF expression stimulated with LPS ··· 20

ABSTRACT

Lipopolysaccharide-induced vascular endothelial growth factor expression in rat lung pericytes

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Vascular endothelial growth factor (VEGF) is a potent angiogenic and vascular permeability factor. Recent studies have shown that the VEGF levels increase in several cell types, e.g., macrophages and smooth muscle cells after lipopolysaccharide (LPS) stimulation, suggesting that it is important in the initiation and development of sepsis. In particular, LPS-regulated contractility in lung pericytes may play an important role in mediating pulmonary microvascular fluid hemodynamics during sepsis. As a first step towards addressing the idea that this study investigated the production of VEGF by rat lung pericytes in response to LPS. LPS was found to enhance VEGF mRNA expression in a concentration-dependent manner peaking 2 h after stimulation in pericytes. VEGF protein levels in conditioned medium and in cell lysate, also increased on increasing LPS and peaked after 24-48 h. LPS also significantly augmented iNOS expression in lung pericytes within 6 h. However, iNOS mRNA induction occurred later than LPS-induced VEGF mRNA increases. Interestingly, attempted inhibition with NF-κB or tyrosine kinase did not suppress LPS-induced augmented VEGF mRNA expression in lung pericytes, although both of inhibitors markedly inhibited LPS-induced

iNOS mRNA expression. SB203580, a p38 MAP kinase inhibitor repressed LPS-induced VEGF mRNA expression. Furthermore LPS stimulated a rapid and sustained phosphorylation of p38 MAP kinase. These results suggest that LPS induces VEGF expression in lung pericytes via p38 MAP kinase, and demonstrates that pericytes produce VEGF in response to LPS stimulation, and that this may be partly mediated by the p38 MAP kinase pathway.

Key Words: lipopolysaccharide, sepsis, vascular endothelial growth factor, pericyte, p38 MAP kinase

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Ⅰ. INTRODUCTION

Lipopolysaccharide (LPS) released during bacterial sepsis causes acute lung injury and acute respiratory distress syndrome (ARDS). LPS is a proximal mediator in the initiation of sepsis and local inflammation, it is also a potent stimulator of other inflammatory mediators, including nitric oxide(NO)[1]. The lung microvasculature is a vulnerable target in sepsis, and ARDS often develops. Pulmonary vascular remodeling and pulmonary hypertension are prominent features of progressive ARDS, and they worsen its prognosis. Moreover, pericytes in the lung circulation contribute to vascular remodeling, regulation, as has been observed in experimental sepsis[2].

In the clinical setting, the loss of capillary permeability regulation in the lungs can lead to extravascular interstitial fluid accumulation, as is observed in ARDS, and is a possible sequela of systemic inflammatory response

syndrome (SIRS)[3,4]. Because pericytes can mediate such changes in capillary permeability, the regulation of the contractilities of lung pericytes may play an important role in pulmonary microvascular fluid hemodynamics during inflammation and sepsis[5].

Previous studies have shown that LPS can control the relaxation of lung pericytes and that neither iNOS inhibitor nor NO donor compounds have any significant effect on pericyte relaxation by LPS stimulation[4]. This ability of LPS to inhibit contractility independently of iNOS has also been demonstrated in lung pericytes derived from iNOS-deficient mice[6]. This finding suggests the presence of an iNOS-independent, but as yet undetermined pathway of lung pericyte contractility regulation.

The vascular endothelial growth factor (VEGF) is a potent angiogenic and vasopermeability factor, which is produced by many types of cells, such as fibroblasts, vascular smooth muscle cells, endothelial cells and macrophages[7-9]. The prominent feature of VEGF production in these cells is that its mRNA levels are increased by hypoxia[9,10].

Recent studies have shown that the VEGF levels are increased in some cells, e.g., macrophages and smooth muscle cells by LPS stimulation, suggesting that it is of importance in the initiation and development of sepsis[11,12]. To clarify the mechanism of VEGF in sepsis, VEGF expression was examined after treating with inhibitors of transcription factors, i.e., NF-κB or p38 MAP kinase. Recently, enhanced VEGF expression has been shown to be mediated via NF-κB, tyrosine kinase or MAP kinase in various cells under different conditions like ischemia[13,14]. However, the direct effects of LPS on VEGF production by pericytes have not been exhaustively studied. In this study, the possibility that LPS, stimulates the expression of VEGF in rat lung pericytes, and its regulatory mechanism have been studied.

Ⅱ. MATERIALS AND METHODS

1. Primary culture of rat lung pericytes

Using a modification of previously described methods[15,16], pericytes were cultured from the lungs of male Sprague-Dawley rats (Samtako Ltd., Osan, Korea), weighing between 250 g and 275 g. The animals were sacrificed by a lethal injection of pentobarbital sodium (120 mg/kg). Each lung was dissected and rinsed with calcium and magnesium-free PBS. To avoid possible smooth muscle cell contamination, only the outer 2 mm peripheral portion of the lung was used. Tissue was minced with scissors and incubated at 37℃ for 30 min in 10 ml of PBS containing 1,000 U/ml collagenase type I, 0.5% BSA, 2 U/ml pronase E, and 0.5 U/ml DNase.

After this initial incubation, the tissue was homogenized with two strokes of a pestle to loosen the microvessel fragments and incubated for a further 10 min. The resulting suspension was filtered through 100 µM nylon mesh, to remove the large tissue fragments, and then washed with DMEM containing 10% fetal bovine serum (FBS) and a 0.5% antibiotic-antimycotic solution.

After washing, the cell pellets were resuspended in 10 ml of red blood cell lysis buffer (Sigma, St. Louis, MO, USA) for 10 min at 4℃, washed twice, and resuspended in uncoated tissue culture dishes in DMEM supplemented with 0.5% antibiotic-antimycotic solution at a cell concentration of 10^6 cells/80 mm dish. The culture medium was changed twice weekly and pericytes were allowed to grow to confluence before being passaged and used in all experiments.

The pericytes demonstrated previously described morphologic characteristics, including the formation of macroscopic mounds at high density, ragged margins, a lack of contact inhibition[3] and were distinguished from other

microvascular cells by their large, irregular shape, dendritic processes and the lack of the hill-and-valley morphology typical of smooth muscle cells at confluence. Pericyte identity was confirmed by the presence of smooth muscle actin, which was observed by indirect immunofluorescent microscopy using monoclonal anti-α-smooth muscle actin and desmin (Sigma, St. Louis, MO, USA)[17]. The cells produced smooth muscle actin, but not desmin, which distinguished them from endothelial cells (Figure 1).

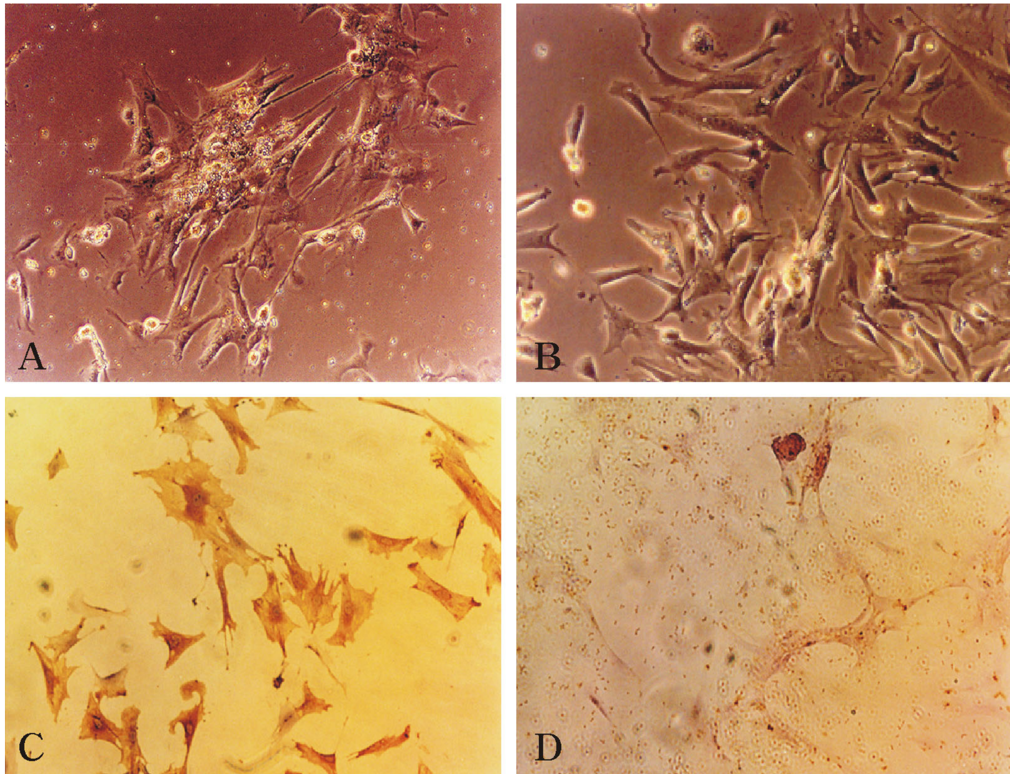

Figure 1. Photomicrographs of rat lung pericytes in culture. LPS did not cause a shape change of cultured pericytes (control dish **(A)** and a dish exposed to 10 µg/ml LPS (**B)** for 24 h). Pericytes were distinguished from other cells of the microvasculature, i.e., from endothelial cells and macrophages, by indirect immunofluorescent microscopy with monoclonal anti-α-smooth muscle actin and desmin. Pericyte identity was confirmed by the presence of smooth muscle actin (**C**) and by the absence of desmin (**D**). All images, were taken at an original magnification of ×400.

2. Experimental design

Rat lung pericytes were stimulated with 10 µg/ml LPS (*Escherichia coli* serotype 055:B5, Sigma, St. Louis, MO, USA) for up to 72 h and the levels of VEGF mRNA and the secretion of VEGF protein were determined. The concentration of LPS in reagents and culture media was measured using an Endospecy kit (Seikagaku Kogyo, Tokyo, Japan), which excludes the effect of exogenous LPS stimulation and excludes bacterial contamination. RT-PCR analyses of VEGF and iNOS mRNA expression in cultured pericytes stimulated with LPS were performed to evaluate the effects of LPS on the levels of VEGF or iNOS mRNA in cultured rat lung pericytes.

In experiments using pharmacologic inhibitors, aimed to investigate the molecular mechanisms underlying the effect of LPS on VEGF, Genistein-a tyrosin kinase blocker (Geni, 100 µM, Sigma, St. Louis, MO, USA), pyrrolidinedithiocarbamate-an NF-κB inhibitor (PDTC, 100 µM, Sigma, St. Louis, MO, USA), and SB203580-a p38 MAP kinase inhibitor (100 µM, Sigma, St. Louis, MO, USA) were used. The cultured cells were pretreated for 2h with each inhibitor before LPS stimulation.

3. RNA extraction and reverse-transcription polymerase chain reaction (RT-PCR)

Total cellular RNA was isolated from the cultured cells with TRIZOL reagent (Gibco BRL, Gaithersburg, MD, USA), according to the manufacturer's instructions. The concentrations of the RNA solutions were determined by spectrophotometry. The quality of the RNA samples was verified by visualizing 28S ribosomal RNA bands after electrophoresis through 1% agarose/3% formaldehyde gels.

RT-PCR was performed using an RT-PCR kit (Takara Bio Inc., Shija,

Japan). An aliquot (1 µg) of the total RNA sample isolated from the rat lung pericytes was reverse transcribed for 30min at 42℃. After being denatured for 12 min at 95℃ and annealed, the reverse transcription products were amplified by two temperature PCR using AmpliTaq Gold DNA polymerase and 38 extension cycles of 30 sec at 63℃, followed by a final extension for 10 min at 72℃[18]. The sequence of the primers used to amplify VEGF and iNOS were as follows[12,19]: VEGF-F (5'-GGT GAGAGGTCTAGTTCCCGA-3'), VEGF-R (5'-CCATGAACTTTCTGCTCTCTT G-3'), iNOS-F (5'-CAAAACACGAGGCTGAGCTGA-3') and iNOS-R (5'-CA GTCCCTTCACCAAGGTGG-3'). The mRNA levels of VEGF, iNOS and 28S were determined using TINA imaging software (Raytest, Straubenhardt, Germany).

4. Enzyme-linked immunosorbent assay (ELISA)

Pericytes were stimulated with 10 µg/ml LPS (*Escherichia coli* serotype 055:B5, Sigma, St. Louis, MO, USA) for up to 72 h and the secretion of VEGF protein were determined. The concentrations of VEGF protein in the conditioned medium or cell lysates were measured using a mouse ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. Briefly, the conditioned media or cell lysates were added to microplate wells coated with VEGF polyclonal antibodies. After incubation for 2h at room temperatue (RT), the wells were washed with the wash buffer supplied in the kit and VEGF antibodies conjugated to horseradish peroxidase and tetramethylbenzidine were then added to each well. After an incubation period of 30 min, the reaction was stopped with hydrochloric acid (supplied in the kit) and the optical density of each well at 450 nm with reference at 550 nm was determined using a SOFTmaxPRO microplate reader (Molecular Devices, Sunnyvale, CA, USA). The VEGF concentration of each sample was calculated using a standard curve obtained by plotting the absorbance of each standard solution.

For the preparation of pericyte-conditioned medium and cell lysates, pericytes were stimulated with LPS, medium was removed and cells were washed twice with serum-free DMEM. The cells were then further incubated in DMEM containing 0.1% human serum albumin (DMEM -HSA), and after 2 h the medium was recovered and subjected to ELISA for VEGF. To determine cell associated VEGF, cells after being stimulated with LPS were lysed by incubating them with cell lysis buffer (20 mM PBS containing 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, and 0.01% protease inhibitor cocktail). The lysate obtained was then passed through a 23G needle, and the cell debris pelleted by centrifugation. The supernatant was then assayed for VEGF using an ELISA kit.

5. Western blot analysis

To identify both the active and inactive forms of p38 MAP kinase in rat lung pericytes, Western blot analysis was performed using specific antibodies recognizing phosphorylated forms of p38 MAP kinase. Cultured pericytes were treated with LPS for up to 120 min at 37℃ in 0.1% BSA. The medium was then removed and the cells were washed with ice-cold PBS, solubilized in lysis buffer (25 mM/L Tris-HCl, pH7.4, 25 mM/L NaCl, 0.5 mM/L EGTA, 1 mM/L sodium orthovanadate, 10 nM/L okadaic acid, 1 mM/L phenylmethylsulfonyl flouride, 2 µg/ml leupeptin, 2 µg/ml aprotinin). Samples were thawed, heated for 5 min at 100℃, and 10 µL aliquots corresponding to 20 µg of protein were loaded into a 12% acrylamide-SDS gel. Blots were transferred to nitrocellulose membranes using an electrophoresis transfer system. Membranes were blocked with 1 × Tris-buffered saline (TBS) containing 5% BSA and 0.1% Tween 20 for 1 h at RT and incubated with a polyclonal rabbit anti-rat phospho-specific or a non-phosphorylated p38 MAP kinase (Transduction Laboratories,

Lexington, KY, USA) in TBS containing 5% BSA overnight at 4℃. Blots were visualized using a HRP-linked anti-rabbit IgG, secondary antibody, and complexes were detected by autoradiography using an ECL chemiluminescence detection system (Amersham Life Science, Little Chalfont, England, UK).

6. Statistical analysis

All data are presented as means±S.D. Standard statistical tests were used to determine differences between groups using Sigma Stat (Jendel Co, San Rafael, CA, USA). Mean values were compared using the unpaired t-test. A P level of < 0.05 was considered statistically significant.

Ⅲ. RESULTS

1. Expression of VEGF mRNA in pericytes stimulated with LPS

The effect of LPS concentration on the expression of VEGF mRNA was examined. Rat lung pericytes were cultured in the indicated concentrations (0.001-100 µg/ml) of LPS for 2 h. The results obtained showed that LPS enhanced VEGF mRNA expression in lung pericytes in a concentration-dependent manner (Figures 2 A, and B). A significant induction of VEGF mRNA levels was evident after treating with 1 µg/ml of LPS and maximal induction was achieved using 100 µg/ml of LPS. The time course of LPS-induced expression of VEGF mRNA was also examined. The results showed that LPS increased VEGF mRNA to a maximal level after 2-3 h of stimulation (Figures 2 C, and D). Thus, LPS was found to be a potent mediator of VEGF gene expression in cultured rat lung pericytes.

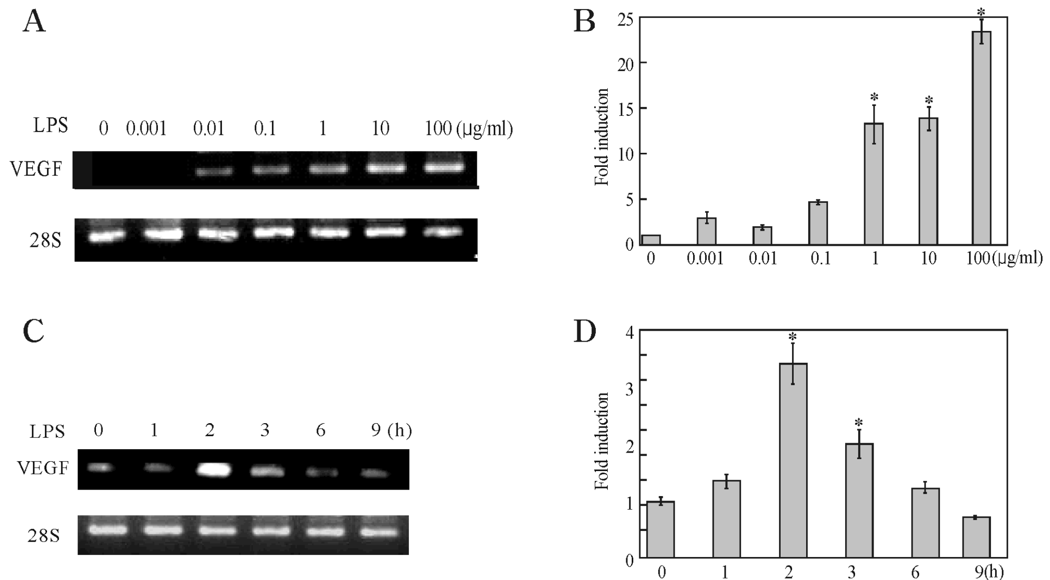

Figure 2. Dose and time response of LPS-induced VEGF mRNA expression in cultured rat lung pericytes. (**A**) Dose dependency. Pericytes were stimulated with various LPS concentrations (0.001-100 μg/ml) concentration for 2 h. (**C**) Time course. Pericytes were stimulated with 10 μg/ml LPS for up to 9 h. The mRNA levels of VEGF and of 28S ribosomal RNAs were semi-quantified by RT-PCR. (**B**), and (**D**) VEGF mRNA and 28S mRNA expression levels were determined using TINA image software. Data were obtained from five independent experiments. VEGF and 28S mRNA expressions in the controls were arbitrarily set as 1.0 each. Means±S.D. are shown. *P <0.05 vs. the controls.

2. Effects of LPS on VEGF protein expression in cultured pericytes

VEGF protein levels, both in conditioned medium and cell lysate, increased with increasing LPS concentration. The average values of VEGF production in the presence of 10 µg/ml LPS were 201±26 pg/ml, for conditioned medium, and 83±21 pg/ml, for cell lysate (n=5, mean± S.D.). Figure 3 summarizes the results of the VEGF protein determinations. The expression of VEGF protein peaked 24-48 h after stimulation with 10 µg/ml LPS and then decreased. The average values of VEGF protein levels after 36 h of stimulation, in the conditioned medium and in cell lysate were 137±35 pg/ml, 75±14 pg/ml, respectively (n=5).

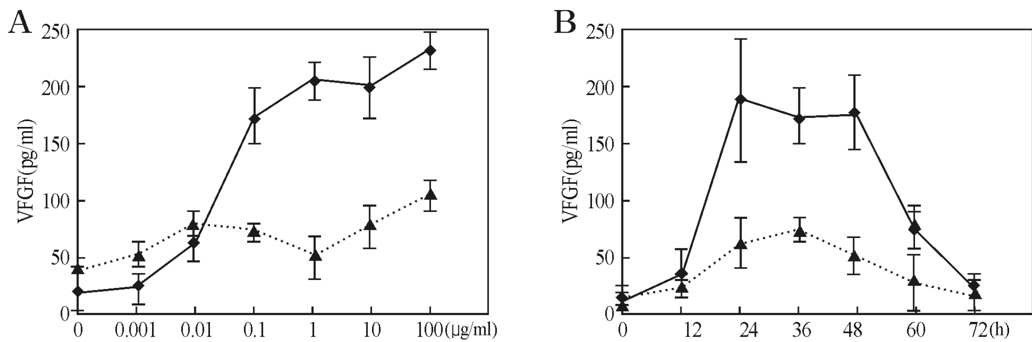

Figure 3. Enhanced VEGF protein expression in rat lung pericytes stimulated with LPS. (**A**) Pericytes were stimulated with 0.001-100 µg/ml LPS for 48 h. VEGF protein concentrations in the conditioned media were measured using a mouse VEGF ELISA kit (R & D SYSTEMS). After stimulation with LPS, the cells were incubated using DMEM for an additional 2 h, and media were recovered and subjected to ELISA (●). The cells were then lysed with cell lysis buffer and the lysates were also subjected to VEGF ELISA (▲). (**B**) Time-dependent expression of VEGF protein in pericytes stimulated with LPS. Graphs represent means±S.D. of five independent experiments.

3. Role of iNOS on LPS-induced VEGF mRNA expression

LPS also significantly augmented iNOS expression in lung pericytes within 6 h (Figure 4). However, the induction of iNOS mRNA took place later than the LPS-induced increases in VEGF mRNA levels.

It was next examined whether various transcription inhibitors could suppress the LPS-induced augmentation of VEGF mRNA expression. Pericytes were incubated for 2 h with or without LPS 10 µg/ml after being pretreated with various agents, i.e., Genistein-a tyrosine kinase inhibitor (Geni, 100 µM), pyrrolidinedithiocarbamate-an NF-κB inhibitor (PDTC, 100 µM), and SB203580-a p38 MAP kinase inhibitor (100 µM). RT-PCR results for VEGF and iNOS mRNA expression were obtained using TINA imaging software.

The inhibition of NF-κB or tyrosine kinase did not suppress the LPS-induced augmentation of VEGF mRNA expression in lung pericytes (Figure 5), although both inhibitors markedly inhibited the LPS-induced expression of iNOS mRNA. These results strongly suggest that the signaling pathways underlying the LPS-induced increase in VEGF mRNA levels may be disassociated from those responsible for iNOS gene induction by LPS in lung pericytes.

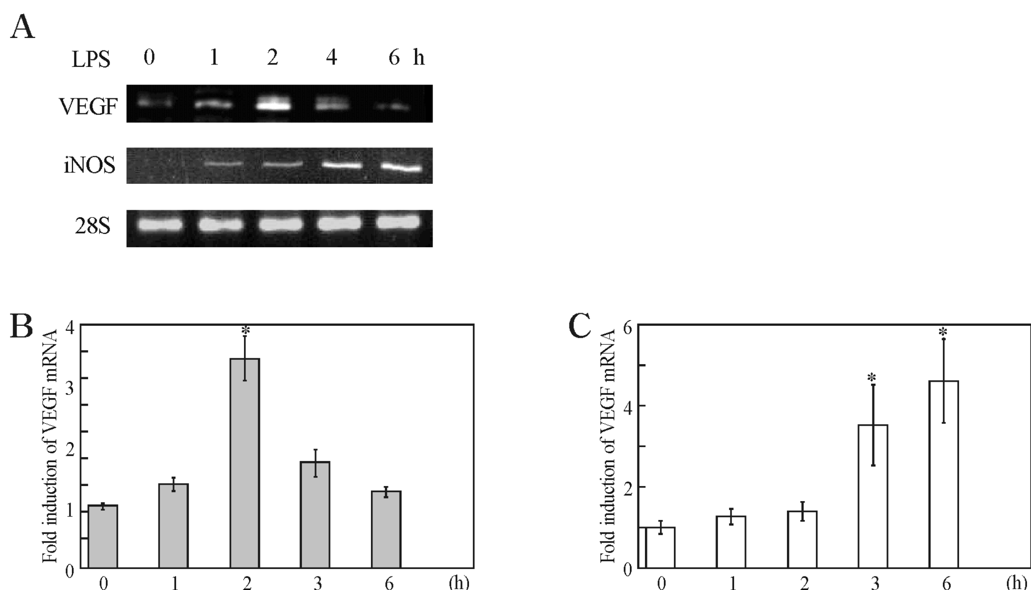

Figure 4. Effects of LPS on the levels of VEGF mRNA and iNOS mRNA in cultured rat lung pericytes. (**A**) Representative results of semi-quantitative RT-PCR of VEGF mRNA and iNOS mRNA expression in cultured rat lung pericytes. A picture of 28S ribosomal RNAs was shown as an internal control. (**B**), (**C**) VEGF mRNA and iNOS mRNA expression levels determined using TINA imaging software. Data were obtained from five independent experiments. The levels of VEGF mRNA and iNOS mRNA in the controls were arbitrarily set as 1.0. Means±S.D. of the experiments are shown. *P <0.05 vs. the controls.

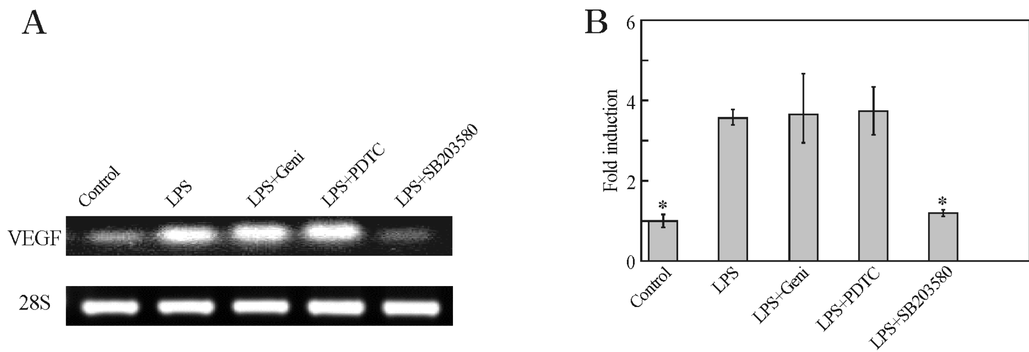

Figure 5. Effects of pharmacological interventions on the LPS-induced expression of VEGF mRNA in cultured rat lung pericytes. (**A**) The results of semiquantitative RT-PCR for VEGF mRNA and iNOS mRNA expressions obtained by incubating pericytes pretreated with various agents prior to being incubated for 2 h with or without LPS 10 µg/ml. The pretreatment included; Genistein-a tyrosine kinase blocker (Geni, 100 µM), pyrrolidinedithiocarbamate-an NF-κB inhibitor (PDTC, 100 µM), and SB203580-a p38 MAP kinase inhibitor (100 µM). A picture of 28S ribosomal RNAs was shown as an internal control. (**B**) VEGF mRNA expression levels were determined using TINA imaging software. Data were obtained from five independent experiments. The levels of VEGF mRNA in the controls were set as 1.0 for each mRNA species and individual experiment. Means±S.D. of the experiments were shown. *P <0.05 vs. cells stimulated with LPS for 2 h.

4. Effect of p38 MAP kinase on LPS-induced VEGF mRNA expression

To identify the signal transduction pathways responsible for the induction of VEGF expression by LPS, we treated rat lung pericytes with the p38 MAP kinase inhibitor SB203580, the tyrosine kinase inhibitor (genistein) and the NF-κB inhibitor (PDTC). The reagent concentrations used in this study have been previously shown to block the effects of agonist in a number of cell types[20].

SB203580, a p38 MAP kinase inhibitor repressed LPS-induced VEGF mRNA expression (Figures 5, and 6A). The activation of p38 MAP kinase was next examined by using antibodies selective for the phosphorylated or non-phosphorylated forms of this kinase by western blot analysis. LPS stimulated a rapid and sustained phosphorylation of p38 MAP kinase (Figure 6B). The phosphorylation of p38 MAP kinase was maximal within 90 min and maintained for 2 h. These results indicate that LPS induces VEGF expression in lung pericytes via p38 MAP kinase.

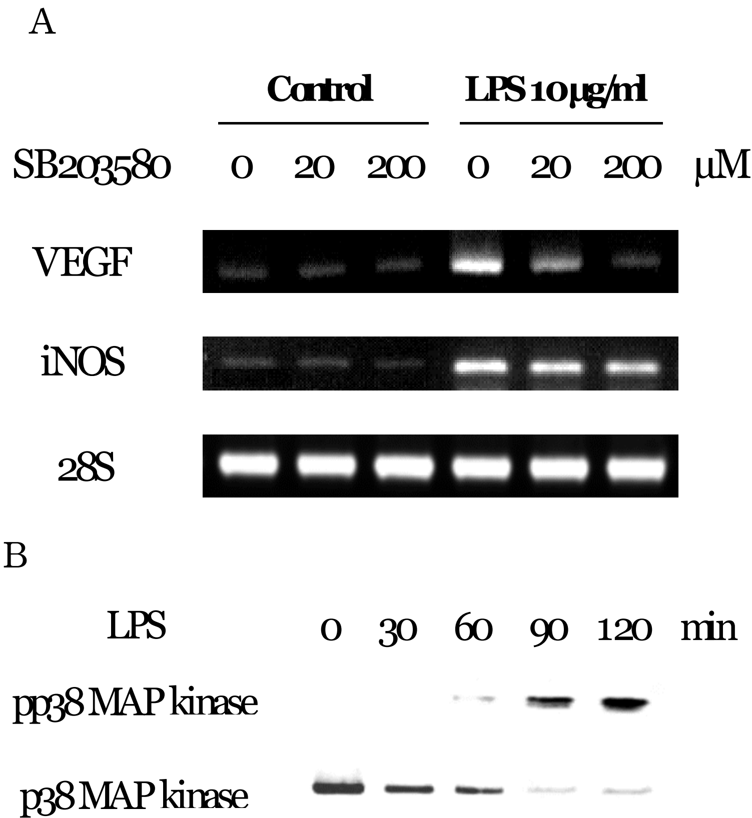

Figure 6. Involvement of p38 MAP kinase in enhanced VEGF by LPS. (**A**) Effects of SB203580, the p38 MAP kinase inhibitor on the LPS-induced enhancement of VEGF mRNA expression. SB203580 was dissolved in DMSO and added, to final concentrations of 0, 20 or 200 μM to the culture medium before 1 h of LPS stimulation. The figures shows representative results of semi-quantitative RT-PCR of VEGF mRNA expressions in cultured rat lung pericytes. A picture of 28S ribosomal RNAs was shown as an internal control. (**B**) The activation of p38 MAP kinase was determined by western blotting with antibodies for the phosphorylated or non-phosphorylated forms of this kinase. These experiments were performed using three different whole-cell lysate preparations.

Ⅳ. DISCUSSION

Without the toxic effects of LPS and secondary cytokine, and the presence of inflammatory mediators, episodes of systemic sepsis might have minimal impact on the lung. However, in many cases of severe sepsis, the lung microvasculature presents a vulnerable target and ARDS often develops[21]. The infusion of LPS in an animal model of sepsis results in pulmonary vascular remodeling, which mimics many of the features of clinical ARDS[22].

This study shows that the expression of VEGF mRNA and the secretion of VEGF protein are both augmented by LPS in rat lung pericytes. Moreover, these increases in VEGF mRNA expression were not suppressed by inhibiting NF-κB or tyrosine kinase activation. The enhancement of VEGF gene expression by LPS in human monocytes and rat macrophages has been previously reported by studies involving transcription factor inhibitors[23.24]. The induction of VEGF gene expression by LPS observed in the present study is accord with other studies of human monocytes and macrophages. However, this study is the first report to describe the possible involvement of VEGF produced by rat lung pericytes in the development of SIRS.

As shown by this study, the LPS-induced augmentation of VEGF mRNA levels also appears to be separate from the iNOS mRNA induction, in terms of the time-course and the effects of pharmacological intervention. Previous studies have shown that the activation of NF-κB and tyrosine kinases are also essential in iNOS induction by LPS also in various cells, like cardiac myocytes[25]. These results are confirmed by the present study, and suggest that the signal pathways underlying VEGF mRNA induction differ from those responsible for iNOS mRNA induction.

The molecular mechanisms responsible for the LPS-induced augmentation of the steady-state levels of VEGF mRNA require elaboration. Hypoxia, a strong inducer of VEGF, was found to increase VEGF mRNA steady-state levels by

activating transcription and enhancing mRNA stability[14]. In the present study, VEGF mRNA levels were rapidly augmented within 2 h of LPS stimulation. In contrast, hypoxia- induced VEGF mRNA increases were reported to take more than 2-3 h in cardiac myocytes[20.23]. Although the half-life of VEGF mRNA has been reported to be short (30-45 min), it seems unlikely that the rapid increase in VEGF mRNA by LPS is wholly due to enhanced mRNA stability, because mRNA stabilization also requires the activation of specific proteins[26]. However, further studies needed to assess the possible contribution made by changes in mRNA stability to LPS-induced VEGF expression.

The ELISA results obtained showed that the VEGF concentrations in the conditioned media were significantly increased at 12-24 h after LPS stimulation ver녀 the controls. Earlier studies have reported that interstitial edema is prominent from 24 h after LPS administration in animals[27]. As VEGF induced by neoplasm or hypoxia was hypothesized to be involved in induction of cerebral edema[11], our present results imply that VEGF may also play a role in development of interstitial edema of the lung and of other tissues in SIRS. Our results concerning the time-course of VEGF secretion may support the above-mentioned hypothesis, because VEGF secretion could precede the occurrence of interstitial edema by LPS.

It is interesting to compare the time sequence of VEGF gene expression and that of p38 MAP kinase activation in LPS-stimulated pericytes.. Western blot analysis using phospho-specific antibodies in LPS-treated whole cell lysate was performed. The results obtained showed that LPS rapidly stimulated the phosphorylation of p38 MAP kinase and that this persisted for 2 h, indicating that p38 MAP kinase activation precedes VEGF gene expression.

VEGF has the ability to promote angiogenesis and to increase microvascular permeability. MAP kinase appears to play a role in both of these biological processes. Many reports have confirmed that p38 MAP kinase has a role in VEGF induced hyperpermeability[28.29]. Taking these findings into consideration,

our results suggest that the phosphorylation of p38 MAP kinase by LPS leads to an increase in VEGF expression, although we did not directly test whether p38 MAP kinase affects VEGF expression. Further studies will clarify this point.

Here, we found that LPS induces VEGF gene expression in rat lung pericytes. Furthermore, these results suggest that p38 MAP kinase is required for LPS-induced VEGF expression and for basal VEGF expression. These findings provide a novel insight into the role of LPS in the pathophysiology of lung pericytes and into the role of VEGF and related proteins such as p38 MAP kinase, in LPS-evoked signaling. Further studies of such VEGF-associated pericyte contractile responses or associated transcription factor are needed to allow us a better understanding the mechanisms involved in the regulation of capillary hemodynamics.

Ⅴ. CONCLUSION

This study demonstrates that LPS increases the expression and the secretion of VEGF in rat lung pericytes. Secreted VEGF may cause pulmonary interstitial edema and systemic edema due to its endocrine action, thus implying that VEGF involved in the pathogenesis of SIRS.

Moreover, these finding suggest that the signaling pathways underlying LPS-induced increases in VEGF expression are distinct from those responsible for iNOS gene induction by LPS, and it appears that the enhancement of VEGF gene expression by LPS is mediated via p38 MAP kinase.

These experiments identify a new role for VEGF in pericytes stimulated by LPS. Its actions may be of great significance in the process of lung microvascular remodeling and hyperpermeability caused by sepsis.

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국문요약

백서 폐주변세포에서 내독소 자극에 의한 혈관내피성장인자의 유전자 발현

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허 애 정

혈관내피성장인자(vascular endothelial growth factor, VEGF)는 혈관형성이나 종양의 전이에 있어서 중요한 역할을 하며 그 밖에 혈관투과성을 조절하는 중요한 물질로 알려져 있다. 혈관투과성에서의 VEGF의 역할에 관하여 최근 활발한 연구가 진행되고 있으며, 그 결과 내피세포, 대식세포, 근세포, 망막주변세포 및 폐주변세포에서 VEGF 유전자 발현을 보고하였다. 특히 다양한 세포들에서 허혈, 고혈당, endothelin-1 및 IL-1 등의 사이토카인이 VEGF 발현을 증가시키는 자극들로 제시되고 있다. 또한 이러한 세포들에서 내독소(lipopolyssacharide, LPS)로 자극시 혈관내피성장인자의 농도가 증가됨을 보고하였는데, 이는 패혈증의 유발에서도 중요한 역할을 할 가능성을 제시하는 결과이다. 그러나 폐주변세포가 패혈증에서 수축력의 변화를 통하여 폐내 미세혈관의 투과성을 증가시켜 패혈증의 다장기부전 및 성인형호흡곤란증후군(adult respiratory distress syndrome, ARDS)의 병인에 기여할 것이라는 여러 연구결과들이 있음에도 아직까지 폐주변세포에서 LPS 자극시 VEGF 유전자 및 단백 발현에 대해 연구한 보고는 없었다. 이에 본 연구에서는 LPS 자극에 따른 폐주변세포의 혈관내피성장인자 생성을 평가하고, 그 기전을 규명하고자 하였다. 폐주변세포를 자극한 LPS의 농도에 비례하여 VEGF mRNA 발현이 증가되었으며, 시간에 따른 변화를 보면 LPS 자극후 2시간에 최대치를 나타내었다. VEGF 단백질 농도는 세포배지 및 세포분해물에서 모두 유의하게 증가되었으며, LPS 자극 후 24-48시간에 최대치를 보였다. iNOS 유전자 발현도 LPS 자극 후 6시간 이내에 의미 있게 증가되었으나 VEGF 유전자 발현보다 이후에 나타났고, iNOS와 연관된 신호전달체계인

NF-κB 및 tyrosine kinase 억제제를 처치하였을 때 LPS에 의한 VEGF 발현은 억제되지 않았다. 이 결과는 LPS 자극에 의한 폐주변세포의 수축력 변화가 iNOS와 무관한 기전에 의한다고 제시한 최근 연구들과 일치한다. 그 외 p38 MAP kinase 억제제 처치 시에 iNOS의 발현은 억제되지 않았으나, VEGF 발현은 유의하게 감소되었으며, LPS 자극시에 p38 MAP kinase의 인산화 반응이 120분 이내에 신속하게 진행됨을 확인하였다. 시간적으로 VEGF 유전자 발현보다 p38 MAP kinase 인산화 반응이 선행하며, 여러 다양한 세포에서 혈관투과성 증가에서의 p38 MAP kinase의 역할이 이미 확립된 점을 고려할 때 VEGF 발현 증가에 p38 MAP kinase가 중요한 역할을 한다는 것을 시사한다고 볼 수 있다. 이상의 결과에서 LPS에 의한 VEGF 유전자 발현은 iNOS와는 무관하며, p38 MAP kinase를 경유하는 기전에 의해 조절될 것으로 생각된다.

핵심 되는 말: 내독소, 패혈증, 혈관내피성장인자, 폐주변세포, p38 MAP kinase