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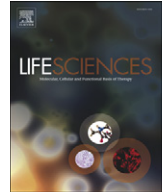

Differential expression of HIF-1α, AQP-1, and VEGF under acute hypoxic conditions in the non-ventilated lung of a one-lung ventilation rat model

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ABSTRACT

Aims: One-lung ventilation (OLV) is a standard practice in thoracic surgery. However, OLV can give rise to arterial hypoxemia. Hypoxia-inducible transcription factor-1 alpha (HIF-1α), vascular endothelial growth factor (VEGF) and aquaporin-1 (AQP-1) may be involved in arterial hypoxemia and contribute to cellular injury. Therefore, in the present study, these moieties were investigated in an OLV rat model.
Main methods: Forty Sprague–Dawley (S–D) rats were randomly divided into four groups: right lung mechanical ventilation for 0.5 h (Group A); 1 h (Group B); 2 h (Group C) and mechanical ventilation of both lungs (control group). Rat lung tissue was examined using electron microscopy. Serum and lung tissue levels of VEGF were measured by ELISA, Western blot analyses were used to detect the protein expression of HIF-1α and immunohistochemical staining and real-time polymerase chain reaction (PCR) were performed to examine protein expression and gene levels, respectively, of VEGF and AQP-1 after hypoxia.
Key findings: Electron microscopy revealed that increased duration of OLV was correlated with greater destruction of the non-ventilated lung. The protein expression of HIF-1α was significantly increased in the non-ventilated lungs of the experimental hypoxia groups (A–C) compared to the control group, whereas VEGF and AQP-1 protein expression and gene levels were decreased in the non-ventilated lungs of the hypoxia groups (A–C) compared to the control group.
Significance: The OLV caused hypoxia in the non-ventilated lung and subsequent injury. The altered expression of HIF-1α, VEGF, and AQP-1 may be involved in the pathological process of lung injury caused by hypoxia.
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Introduction

One-lung ventilation (OLV) is a standard practice in thoracic surgery. It improves access to the operative field, isolates and protects the lungs during the procedure, and expedites surgery. However, hypoxemia, as a result of low blood oxygen, can occur in the non-ventilated lung causing disturbances in lung ventilation/perfusion functions. These events can precipitate acute lung injury (ALI), a major and serious complication of OLV [10], occurring in more than 1% of patients that undergo thoracic surgery [18].

Ultimately, ALI is an uncontrolled inflammatory response caused by extensive damage in pulmonary vascular endothelial cells and alveolar epithelial cells [4]. Tissue inflammation, increased vascular permeability, and plasma leakage can all occur as a result of ALI [13,20], which manifests as diffuse lung exudation and severe hypoxemia. These symptoms are characteristic of an early stage of acute respiratory distress syndrome (ARDS).

Hypoxia-inducible transcription factor-1 alpha (HIF-1α) is one of the most important factors in the response to hypoxia as it assists tissues to adapt to the ALI-induced hypoxic conditions and minimizes tissue damage [7,14,22]. Vascular endothelial growth factor (VEGF) is a glycosylated mitogen with various functions including: increasing vascular permeability; inducing endothelial cell growth; mediating cell migration and inhibiting apoptosis. It has been shown that VEGF promotes alveolar epithelial cell proliferation and accelerates restoration of epithelial damage [26]. In addition, VEGF has vasodilatory functions, as it promotes pulmonary edema following alveolar capillary membrane damage as a result of ARDS [11]. The overall significance of the role of VEGF in OLV remains, however, unclear [7].

Aquaporins (AQPs) were first identified in mammals as proteins capable of increasing plasma membrane water permeability, thus facilitating rapid fluid movement [25]. Diverse lung pathologies are characterized by disruption in fluid transport, including congestive heart failure, respiratory distress syndrome and pulmonary edema arising from injury or infection [5]. Aquaporin-1 (AQP-1) is primarily expressed on the surface of pleural visceral capillary endothelial cell cavity membranes and visceral pleural mesothelial cell membranes [19]. Alveolar capillary permeability is reportedly decreased by 10-fold in AQP-1 and AQP-5 knockout mice and decreased 16-fold in AQP-1 and AQP-4

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knockout mice, indicating an important role of AQP-1 in the regulation of lung water balance [24]. Abreu-Rodríguez et al. [2] showed that HIF-1α regulates an AQP-1 promoter mutation in a hypoxic cell culture environment. However, few reports have described the expression and the potential role of HIF-1, AQP-1 and VEGF in OLV.

To address this, the current study investigated HIF-1α, AQP-1 and VEGF expression in an OLV rat model exposed to hypoxic conditions for varying lengths of time. It was hypothesized that an increase in the length of time of OLV would be associated with increased hypoxic lung injury in the non-ventilated lung, altered expression of HIF-1α, AQP-1 and VEGF and that the mutual relationship of these moieties may play an important role in hypoxic lung injury.

Materials and methods

Animal model of OLV

Forty male Sprague–Dawley (S–D) rats (200–250 g, Animal Centre, Guangxi Medical University, China), were randomly divided into four groups — three treatment groups and one control group. The three treatment groups were given regional anesthesia, intraperitoneal (i.p.) injection of 10% chloral hydrate (4.5 mL/kg) and their right lungs alone were mechanically ventilated for 0.5 h (Group A), 1 h (Group B), or 2 h (Group C). The control group was given the same regional anesthesia, and both lungs were mechanically ventilated. Mechanical ventilation was applied using a RSP1002-type small animal ventilator with a respiratory ratio (inspired to expired air) of 1:1 and a breathing frequency of 80 breaths/min. The tidal volume was 10 mL/kg and the fraction of inspired oxygen was 100%. Lung collapse and expansion were observed and noted. At the end of each hypoxic period, rats were sacrificed by cervical vertebra dislocation and 2 mL of blood was collected from the left ventricle of each rat. Blood samples were centrifuged at 1509 ×g for 15 min. Serum was removed from the samples and stored at −80 °C until further analyses. Portions of the lower lobe of the non-ventilated (left) lung were excised from rats in all treatment groups and the ventilated left lung of the control group. The lung tissue was divided into three portions. One portion was snap frozen and stored at −80 °C for Western blot (WB) and quantitative PCR (qPCR) analyses, another portion was fixed for microscopy and a third portion was used to perform an enzyme-linked immunosorbent assay (ELISA) for the detection of VEGF. No rat mortality was recorded during the experimental procedure. All animal studies were carried out in accordance with the animal's guidelines of the University Institutional Animal Care and Use Committee.

Hematoxylin and eosin (H&E) staining

Rat lung tissues were fixed in 4% (w/v) paraformaldehyde and embedded in paraffin. Sections were prepared and evaluated by experienced pathologists, blinded to the experimental treatment conditions.

The 4 micron thick slices were deparaffinized by xylene and hydration, stained for 5 min with hematoxylin, differentiated for 30 s by hydrochloric acid ethanol, soaked for 15 min in water and stained for 2 min with eosin. The H&E stained slices underwent conventional dehydration, transparent and sealing.

Electron microscopy

Rat lung tissues were cut into 1-mm^3 sections, washed with cold phosphate-buffered saline (PBS) solution and fixed with 2% glutaraldehyde (pH 7.4) for 30 min. Tissues were then rinsed with PBS three times, dehydrated using a series of increasing concentrations of acetone (50, 70, 90, and 100%), and embedded in resin for 30 min at room temperature (RT). Ultrathin sections were prepared and stained using lead acetate uranium and examined under an electron microscope (H-500, Hitachi; Japan).

VEGF and AQP-1 immunohistochemical staining

Lung tissue sections from the lower lobe of the unventilated (left) lung were prepared as described for the H&E staining, and then deparaffinized and dehydrated. Endogenous peroxidase activity was blocked using a 10 min treatment of 3.0% hydrogen peroxide (H_2O_2). After antigen retrieval with citrate buffer (pH 6.0), sections were immediately blocked using goat serum for 30 min at 37 °C, then incubated with anti-VEGF (Abcam, HK, Cat# ab1316, 1:1000 dilution) or anti-AQP-1 primary antibody (Abcam, HK, Cat# ab15080,1:1000 dilution) for 90 min at RT. Slides were then washed three times with PBS for 3 min, followed by a 60 min incubation with biotinylated secondary antibody (Abcam, HK, Cat# ab6940; anti-rabbit, 1:2000 dilution). Following this, diaminobenzidine (DAB) was used to detect the secondary antibody, indicated by dark brown staining. Slides were washed with tap water, stained with H&E, and dehydrated. Coverslips were mounted using neutral resin. As a staining control, PBS was used in place of primary antibody. The expression of each protein was assessed by the intensity of staining within each microscopic field. The images were captured by a digital camera connected to a microscope (BX512DP70; Olympus). Five fields were selected, and the mean optical density was measured using Image-Pro Plus 5.0 software (Media Cybernetics, American).

ELISA detection of VEGF

To measure the expression of VEGF in serum and lung tissue, a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) was performed. Fresh lung tissue, harvested as previously described, was pulverized, placed into individual test tubes and ice cold saline added. An ELISA was performed using a kit (RayBiotech, Atlanta, GA, USA), in accordance with the manufacturer's instructions.

Quantitative real-time RT-PCR analysis

Total lung tissue RNA was isolated using TRIzol reagent (Invitrogen, California, USA). RNA was reverse transcribed to single-strand cDNA using a Revertaid First Strand cDNA Synthesis kit (MBI Fermentas, Canada) according to the manufacturer's protocol. Reverse transcription was carried out using a PCR kit (Roche, Basel, Switzerland) in a 20 μL reaction containing 2 μg RNA, 50 mM KCl, 50 mM Tris/HCl, 4 mM $MgCl_2$ and 10 mM of dNTPs, oligo-(dT) primers, RNAse inhibitor and MuLV reverse transcriptase. The reaction mixture was incubated for 5 min at 37 °C, 60 min at 42 °C, and then heated to 70 °C for 5 min in a thermocycler (MBI Fermentas, Canada). Quantitative real-time PCR was conducted with a PTC 200 real-time PCR reactor (MJ Research, Fremont, CA, USA) for SYBR green PCR master mix (Takara, Shiga, Japan) according to the manufacturer's protocol. Primers were designed according to the respective gene sequences using Primer 3 software and synthesized by Sangon Biotech (China). The PCR primers are shown in Table 1. The PCR conditions were 94 °C for 3 min, 94 °C for 45 s, 59.2 °C (VEGF) or 61.8 °C (AQP-1) for 1 min and 72 °C for 30 s, for a total of 35 cycles, with a final extension for 10 min at 72 °C. The amplified PCR products were electrophoresed on a 1.5% agarose gel. Relative gene expression

Table 1
Primers used in this study.

Name	Primer sequence
HIF-1α sense	5′-ACAGCACATTCACAGCTCCCCA-3′
HIF-1α anti-sense	5′-TGTGGCTACCATGTACTGCTGGC-3′
AQP-1 sense	5′-CTCACCCGCAACTTCTCAAAC-3′
AQP-1 anti-sense	5′-CATCCAGGTCATACTCCTCCAC-3′
VEGF sense	5′-GGAGTACCCCGATGAGATAGAGT-3′
VEGF anti-sense	5′-CTATGTGCTGGCTTTGGTGAG-3′
β-Actin sense	5′-TCAGGTCATCACTATCGGCAAT-3′
β-Actin anti-sense	5′-AAAGAAAGGGTGTAAAACGCA-3′

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