Original Article

Effects of sevoflurane on pulmonary cytosolic phospholipase A2 and clara cell secretory protein expressions in rabbits with one-lung ventilation-induced lung injury

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Abstract: Objective To investigate the effects of sevoflurane on cytosolic phospholipase A2 (C-PLA2) and clara cell secretory protein (CCSP) in lung tissues of rabbits with one-lung ventilation (OLV)-induced lung injuries. Methods Thirty-six healthy Japanese white rabbits were randomized into sham-operated group, OLV group, and OLV plus sevoflurane group subdivided into 4 subgroups with sevoflurane concentrations of 1%, 2%, 3% and 4%. CCSP and C-PLA2 mRNA and protein expressions in rabbit lung tissues were detected by Western blotting and real-time PCR, and the content of arachidonic acid (AA) was measured using ELISA. The severities of the lung injury were evaluated according to lung wet/dry weight (W/D) ratio and histological scores. Results In the OLV group and OLV+ sevoflurane groups, pulmonary CCSP expressions were significantly lower, while C-PLA2 expression, lung W/D ratios and lung histological scores were significantly higher than those in the sham-operated group (P<0.05). Compared with OLV group, the OLV+ sevoflurane groups showed significantly increased expressions of CCSP and reduced C-PLA2 expression, lung W/D ratios and histological scores (P<0.05). In the 4 OLV + sevoflurane groups, CCSP expressions underwent no significant changes as sevoflurane concentration increased, but C-PLA2 expressions, lung W/D ratios and histological scores all decreased gradually as the concentrations of sevoflurane increased (P<0.05). Conclusion OLV can result in down-regulated CCSP expressions and up-regulated C-PLA2 expressions in rabbit lung tissues. Sevoflurane can protect against OLV-induced acute lung injury possibly by inhibiting C-PLA2 expression via up-regulation of CCSP expressions or through other mechanisms resulting in down-regulated expression of C-PLA2.

Key words: sevoflurane; one-lung ventilation; acute lung injury; cytosolic phospholipase A2; clara cell secretory protein

INTRODUCTION

Studies have shown that one-lung ventilation (OLV) may result in acute lung injury (ALI) [1,2], in which lung inflammatory responses in the absence of infection are considered to be one of the primary mechanisms [3]. As a widely used volatile inhaled anesthetic, sevoflurane was shown to alleviate OLV-induced lung inflammatory responses [4], but the underlying mechanism has not been fully understood.

Arachidonic acid (AA) and its metabolites can lead to inflammatory responses by increasing vascular permeability and recruiting neutrophils [5]. Phospholipase A2 (PLA2) is a key rate-limiting enzyme for AA release [6]. In the phospholipase A2 (PLA2) family, cytosolic PLA2 (C-PLA2, relative molecular mass of 85 000) is recognized as a potentially important pharmacological target for the control of inflammatory diseases [7] and has been shown to mediate inflammatory responses in ALI caused by mechanical ventilation [8]. Clara cell secretory protein (CCSP), a protein with a lung tissue specificity, has been identified as an endogenous PLA2 inhibitor with anti-inflammatory effects against ventilator-induced lung injury by inhibiting C-PLA2 [9].

We therefore hypothesized that OLV results in ALI by down-regulating pulmonary CCSP and up-regulating C-PLA2 expressions to increase AA production, and sevoflurane can protect against OLV-induced ALI by reversing the adverse effects of OLV.

MATERIALS AND METHODS

Animal grouping

Thirty-six healthy Japanese white rabbits (weighing 2.2-2.5 kg) of either sex were purchased from the Experimental Animal Center of Kunming Medical University. The rabbits were randomized into three groups, namely the sham-operated group (n=6), OLV group (n=6), and OLV plus sevoflurane group (n=24). The sevoflurane groups were further divided, according to the concentration (volume percentage) of sevoflurane administered, into 1%, 2%, 3% and 4% groups (n=6).

Sham operations were performed by exposing the trachea, left common carotid artery and right external
jugular vein, and an endotracheal tube (inner diameter of 2.0 mm) was placed via tracheotomy between 2 and 3 tracheal rings. In the OLV groups, OLV was achieved by advancing the tracheotomy tube into the right main bronchus. Mechanical ventilation was performed by OLV for 2 h followed by two-lung ventilation (TLV) for 1 h with identical ventilator (Datex Ohmeda, Aestiva/5 7900, USA ) settings: inspired oxygen fraction (FiO₂) = 1.0, tidal volume (VT)=20 ml/kg, respiration rate (RR)=30 breaths/min, and inspiratory : expiratory ratio (I/E)=1:2.

**Anesthesia and intraoperative detections**

The mean arterial pressure, end tidal CO₂ and sevoflurane concentrations were monitored continuously and maintained within the normal ranges. Ringer’s solution (10 ml·kg⁻¹·h⁻¹) was continuously infused through the right external jugular vein catheter. All the rabbits were anesthetized with intravenous pentobarbital sodium (30 mg/kg) during the sham operations. Anesthesia was maintained by continuous infusion of remifentanil at a rate of 1 μg·kg⁻¹·min⁻¹ and intermittent administration of vecuronium (0.1 mg/kg per 30 min) during OLV and TLV.

**Lung W/D ratio**

The right lower lobe was excised and the wet weight was recorded. The lung was then desiccated at 80 °C for 72 h before dry weight was measured to calculate the W/D ratio.

**Lung histological score**

The right upper lobe was sectioned and stained with hematoxylin and eosin. The slides were viewed by a blinded pathology technologist using light microscopy for histological evaluation of the injury.

**Quantification of CCSP and C-PLA₂ mRNA expressions**

The total RNA was extracted from the right middle lobes using Trizol RNA extraction kit (Roche, 4896866001). The first-strand cDNAs were synthesized using RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, K1612) according to the manufacturer’s protocol. PCR was performed with a reaction volume of 25 μl using a BioEasy SYBR Green I Real Time PCR Kit (Roche, 04913850001) according to the manufacturer’s instructions. The results obtained from real-time RT-PCR were quantified by the 2⁻ΔΔCt method. The sense (S) and antisense (AS) of the primers (5’-3’) used for each target mRNA were:

- **β-actin**: CATCCTGACGCTCAAGTA/GTTGTAGAGAGTGTGTTG;
- **CCSP**: CACCAAGGCTTCCAACCT/GGCAGATGTCCGAGAGAG;
- **C-PLA₂**: CCTAATCATGTTGGAAGATA/ATGGTTGCTTGAGAAATA.

Western blotting for CCSP and C-PLA₂ protein expressions

Freshly frozen samples of the lung tissues from the rabbits were homogenized in 1 ml pre-cooled RIPA lysis buffer (Beyotine Institute of Biotechnology, P0013C) and then 15 μl protease inhibitor cocktail (Roche, 4693132001) was added. The protein concentrations were determined using the BCA kit (Roche, P0010). For Western blotting, the samples were incubated overnight at 4 °C with the primary antibodies (CCSP: Abcam ab50711; C-PLA₂: Abcam, ab73406), and then at room temperature with the secondary antibody (Abmart, M21003) for 2 h. The proteins were detected by enhanced chemiluminescence (BIO-RAD), and the band intensities were quantified using Image J software.

**Determination of pulmonary AA content with ELISA**

The content of AA in lung tissues was measured using a commercially available sandwich ELISA kit (Biosynthesis Biotechnology Co., LTD) according to the manufacturer’s instructions.

**Statistical analysis**

The quantitative data are presented as Mean±SE. One-way analysis of variance (ANOVA) with repeated measures followed by Fisher’s least significant difference post hoc test was used as indicated for comparison between the groups. A P value less than 0.05 was considered to indicate a statistically significant difference.

**RESULTS**

**CCSP and C-PLA₂ mRNA and protein expressions**

CCSP expressions (at both mRNA and protein levels if not indicated otherwise in the text, which also applies to C-PLA₂ expression) in the lung tissue decreased significantly while those of C-PLA₂ increased in both the OLV group and OLV + sevoflurane groups as compared with those in the sham-operated group (P<0.05). Compared with the OLV group, the OLV + sevoflurane groups showed significantly higher CCSP but lower C-PLA₂ expressions (P<0.05). In the 4 OLV + sevoflurane groups, increased sevoflurane concentrations did not cause significant changes in the levels of CCSP expression but resulted in gradually decreased C-PLA₂ expressions in the lungs (P<0.05, Fig.1).

**Pulmonary AA content, W/D ratio and histological scores**

In both the OLV group and OLV + sevoflurane group, the pulmonary AA contents, lung W/D ratios and histological scores all increased significantly as compared with those in the sham-operated group (P<0.05). Compared with those in the OLV group, AA
contents, lung W/D ratios and histological scores in the 4 OLV + sevoflurane subgroups all decreased gradually as the concentrations of sevoflurane increased ($P<0.05$, Fig.2).

**Lung histology**

No significant pathological changes were observed in the sham-operated group except for mild inflammatory reaction and capillary dilatation in some areas of lung tissues (Fig.3A1-2). In the OLV group, the lung tissues presented with severe hyperemia and hemorrhage, alveolar wall thickening and exudation, with markedly red blood cell and inflammatory cell infiltration in the alveolar space (Fig.3B1-2). In the 4 OLV + sevoflurane subgroups, these pathological changes in lung tissues were alleviated gradually as the concentration of sevoflurane increased (Fig.3C1-2 to F1-2).

**DISCUSSION**

Our first finding in this study is that OLV can lead to ALI by significantly reducing pulmonary CCSP expressions and increasing C-PLA$_2$ expression and AA contents in the lung tissue. As a strong endogenous inhibitor of PLA$_2$, CCSP can inhibit C-PLA$_2$ [12] to reduce AA production in the lung. We speculate that the up-regulated expressions of C-PLA$_2$ is associated with down-regulated expressions of CCSP induced by OLV, which increases AA production to result in ALI.

The second finding in this study is sevoflurane administrations can alleviate ALI and causes increased expressions of pulmonary CCSP and decreased expressions of C-PLA$_2$ with reduced AA content in the lung under OLV. This finding suggests that the inhibition of C-PLA$_2$ expression by up-regulating CCSP expression may be one of the mechanisms underlying the protective effects of sevoflurane against OLV-induced ALI. But as the increases in sevoflurane concentration decreased pulmonary C-PLA$_2$ expression and ameliorated the lung injury, the expressions of CCSP did not undergo any obvious changes. This
Studies have shown that C-PLA$_2$ activation was related to the up-regulation of nicotinamide-adenine dinucleotide phosphate oxidase (NADPH oxidase), mitogen-activated protein kinase (MAPKs), activator protein-1 (AP-1), nuclear factor-kB (NF-kB) and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) [13-14]. Interestingly, mechanical ventilation also activated these factors [15-20], which could all be inhibited by sevoflurane [21-25]. We thus speculate that the inhibitory effects of sevoflurane on C-PLA$_2$ may be mediated by decreasing the activities of these factors. In addition, one possible explanation for the unchanged expression pattern of CCSP in response to increasing concentrations of sevoflurane lies in the plateau effects of sevoflurane on CCSP when sevoflurane concentration exceeds 1%, although the minimal concentration of sevoflurane in our study was below the commonly used concentration in clinical practice. But the exact mechanisms still need to be elucidated by further research.

In summary, we identify for the first time that OLV induces ALI possibly by down-regulating CCSP and up-regulating C-PLA$_2$ expressions in the lungs, and sevoflurane may protect against OLV-induced ALI by reversing this effect. Further studies are required to fully understand the influences of sevoflurane on OLV-induced ALI after removing the role of CCSP and explore the other possible mechanisms of sevoflurane-mediated C-PLA$_2$ regulations.

REFERENCES


