INTRODUCTION

Studies have shown that one-lung ventilation (OLV) can cause pulmonary inflammation responses [1-2]. Specific eicosanoids, lipid mediators derived from arachidonic acid (AA), are known to contribute to the initiation of inflammation [3]. Blocking the pathways of AA metabolism, therefore, may alleviate the lung injury induced by OLV.

In mammals, AA can be metabolized mainly by two pathways, namely the cyclooxygenase (COX) pathway which generates classical prostaglandins (PGs), prostacyclin (PGL2) and thromboxane (TXA2), and the lipoxigenase (LOX) pathway which generates leukotrienes (LTs) and lipid peroxides. There are two different COX isoforms, COX1 and COX2, and the latter is an inducible isoform that is found and expressed mainly in inflammation responses [4]. Among the various end products derived from the sequential metabolism of AA via COX2, PGL2, which is a potent vasodilator and an inhibitor of platelet aggregation produced mainly by vascular endothelial cells, has been implicated as the PGs most responsible for inflammation [5]. As another product of the sequential metabolism of AA, TXA2 is generated mainly by platelet COX1 pathway and executes the functions of both a potent vasoconstrictor and a stimulator of platelet aggregation [6]. In physiological conditions, the levels of TXA2 and PGL2 maintain a relative equilibrium state, and breaching this balance between them results in disease just as the lessons learned from the specific COX2 blocking agent that led to cardiovascular complications [5]. This suggests that maintaining the equilibrium state of TXA2 and PGL2 is more important than simply manipulating either of them in the disease process. Therefore, it is more reasonable to use the ratio between PGL2 and TXA2 to reflect the severity of the disease than use either of them as a single indicator.

Leucotrienes synthesized via the 5-LOX pathway play a major part in the inflammatory process [6]. The final and biologically active metabolites of the 5-LOX cascade are LTC4, LTD4, LTE4, and LTB4; the first three mainly cause bronchospasm and mucosal secretion, and LTB4 is a potent stimulator of leucocytes activation, allows these cells to adhere to the vascular endothelium, and elicits chemokinetic and chemotactic responses [7-10]. During a brief exposure to LTB4, polymorphonuclear
leucocytes (PMN) are predominantly recruited. Robertson et al. and Caironi et al. showed that inhibition of COX2 and 5-LOX expressions exerted an anti-inflammatory effect in ventilator-induced lung injury. Although some studies showed that sevoflurane, a commonly used anesthetic in clinical practice, played an anti-inflammatory role in OLV, the effects of sevoflurane on COX2 and 5-LOX in rabbit lung tissues with OLV-induced injuries are still unclear. We hypothesize that sevoflurane may also protect against OLV-induced acute lung injury (ALI) by modulating AA metabolism through COX2 and 5-LOX pathways.

MATERIALS AND METHODS

Animals and grouping

Eighteen healthy Japanese white rabbits of either sex weighing 2.2–2.5 kg were purchased from the Experimental Animal Center of Kunming Medical University (Animal Certificate of Conformity: 0020946; animal license number: sexk (Yunnan) 2011–0004). The rabbits were randomized equally into 3 groups, namely the sham-operated group (S group), OLV group (O group), and OLV plus sevoflurane group (OS group). In S group, the rabbits received sham operations by exposing the trachea, left common carotid artery and right external jugular vein, and placement of endotracheal tube (inner diameter of 2.0 mm) through tracheotomy between 2 and 3 tracheal rings. In O group, OLV was achieved through advancing the tracheotomy tube into the right main bronchus in rabbits, and mechanical ventilation was performed by OLV for 2 h followed by two-lung ventilation (TLV) for 1 h. The ventilator settings (Datex Ohmeda, Aesitiva/5 7900, USA) in OLV and TLV were identical with an inspired oxygen fraction (FiO2) of 1.0, tidal volume (VT) of 20 ml/kg, respiration rate (RR) of 30 min–1, and inspiratory/expiratory (I/E) ratio of 1:2. In OS group, 2.5% sevoflurane was administered during mechanical ventilation with the same settings as in O group.

Anesthesia and intraoperative detections

All the rabbits were anesthetized with pentobarbital sodium (30 mg/kg) via the ear marginal vein during the sham operations. Maintenance of anesthesia in O group and OS group was achieved 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). Invasive blood pressure, end tidal CO2 and sevoflurane concentrations were continuously monitored and maintained within the normal ranges or at the preset concentration.

Determining pulmonary contents of LTB4, PGI2, 6-keto-PGF1α, TXA2, and TXB2

Due to the short tissue half-life of TXA2 (30 s) and PGI2 (3 min)6, the quantities of their stable metabolites, TXB2 and 6-keto-PGF1α, respectively, were determined to represent their values. Enzyme-linked immunosorbent assay (ELISA) kits (Biosynthesis Biotech Co., LTD, China) were used to detect the amounts of LTB4, 6-keto-PGF1α and TXB2, in the lungs of the rabbits according to the manufacturer's instructions.

Western blotting for determining pulmonary COX2 and 5-LOX protein contents

The right middle lobes of the rabbit lungs were sampled (weighing about 100 mg) and quickly placed on ice. One milliliter of pre-cooled RIPA lysis buffer (Beyotime Institute of Biotechnology, P0013C) and 15 μl protease inhibitor cocktail (Roche, 4693132001) were added. The soluble proteins were isolated by centrifugation at 13 000 r/min for 30 min, and the protein concentrations in the supernatant were determined using BCA kit (Beyotime Institute of Biotechnology, P0010). For Western blotting, the samples were boiled in 6 × loading buffer, and the proteins were separated by SDS-PAGE and transferred to PVDF membranes (0.45 μm, Millipore). The membranes were then blocked with 5% bovine serum albumin (BSA) at room temperature for 60 min, rinsed with TBST for 3 times, incubated overnight at 4 °C with the primary antibodies of COX2 (Biosynthesis Biotech Co. Ltd, bs-0732R, dilution 1:300) and 5-LOX (Biosynthesis Biotech Co. Ltd, bs-0526R, dilution 1:200), and then at room temperature with the secondary antibody (Abmart, M21003, dilution 1:3000) for 2 h. The proteins were detected by enhanced chemiluminescence (BIO-RAD), and the band intensities were quantified using Image J software.

Quantitative real-time PCR for COX2 and 5-LOX mRNA expressions

Under sterile conditions, the total RNA was extracted from the right middle lobe tissues (each sample weighing about 200 mg) using Trizol RNA extraction kit (Roche, 4896866001), and the concentrations and purities of the total RNA were measured. The first strand cDNAs were synthesized using RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, K1612) according to the manufacturer’s instructions. Real-time RT-PCR amplifications of the samples in triplicate were performed using the SYBR Green PCR Master Mix (Applied Biosystems) in a total reaction volume of 25 μl containing distilled water (9.5 μl), SYBR Green Master (12.5 μl) (Roche, 04913850001), 10 μmol/L upper-stream primer (1 μl), 10 μmol/L downstream primer (1 μl) and template cDNA (1 μl). All the PCR assays were run in the ABI Prism 7300 Sequence Detection System at 95 °C for 3 min, 95 °C for 15 s, 62 °C for 30 s, 72 °C for 30 s, followed by 40 cycles of 95 °C for 15 s, 62 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. The primers used for PCR amplification were synthesized by Invitrogen Corporation (Tab.1).
Lung histological scores

A sample of the right upper lobe was sectioned for histological examination. The sections were fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. The slides were viewed by a blinded pathologist under light microscopy for histological evaluation of the lung injury according to the criteria proposed by Webb et al.15.

Lung wet/dry weight (W/D) ratio

At the end of the experiments, the right lower lobe was excised. The wet weight was recorded, and the lung was desiccated at 80 °C for 72 h for measurement of the dry weight to calculate the W/D ratio.

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

Pulmonary expressions of COX₂ and 5-LOX

In both O group and OS group, COX₂ and 5-LOX expressions (at both the protein and mRNA levels if not indicated otherwise) were significantly increased compared with those in S group (P<0.05). COX₂ and 5-LOX expressions were significantly lower in OS group than in O group (P<0.05, Fig.1).

Pulmonary contents of LTB₄, 6-K-PGF₆ (PGI₂), TXB₂ (TXA₂) and 6-K-PGF₆/TXB₂

Compared with those in S group, the pulmonary contents of LTB₄, TXB₂ and 6-K-PGF₆ increased while 6-K-PGF₆/TXB₂ ratios decreased significantly in O and OS groups (P<0.05). The contents of LTB₄, TXB₂ and 6-K-PGF₆ were lower but 6-K-PGF₆/TXB₂ ratio was higher significantly in OS group than in O group (P<0.05, Fig.2).

Lung W/D ratio and histological scores

In both O group and OS group, lung W/D ratios and histological scores were significantly increased as compared with those in S group (P<0.05). The lung W/D ratio and histological scores were significantly lower in OS group than in O group (P<0.05, Fig.3).

Lung histology under light microscopy

No significant pathological changes were observed in S group except for mild inflammations and capillary dilatation in some areas of the lung tissues (Fig.4A1-2). Serious hyperemia and hemorrhage in the lung tissues, thickening and exudation of the alveolar wall, marked red blood cell and inflammatory cell infiltration in the alveolar space were found in O group (Fig.4B1-2). These pulmonary histopathological changes were alleviated significantly in OS group (Fig.4C1-2).

DISCUSSION

Our data showed that COX₂ and 5-LOX expressions increased significantly along with LTBr, TXAr and PGI₂ in the rabbit lungs after OLV, which led to serious lung injuries. These results suggest that OLV activates the COX₂ and 5-LOX pathways and then results in increased metabolites of the two pathways.

We also found a significantly decreased PGI₂/TXA₂ ratio in the lung tissue after OLV, which can be the results of multiple factors. LTBr generated through the activation of 5-LOX pathway induced by OLV can activate a large amount of inflammatory cells, which adhere to the vascular endothelium and cause vascular endothelial cell damages. The vascular endothelial damages lead to platelet aggregation and activation, and then the production of numerous TXAr. The generated TXAr in return, causes further platelet aggregation and activation, thus completing a vicious cycle to lead to drastically increased TXA₂ contents in the lung tissue. Conversely, OLV-induced damages of the vascular endothelial cells, which are the major source of PGI₂ synthesis, cause a reduction in PGI₂ production. Therefore, even if OLV increases COX₂ expression, PGI₂ production is in disadvantage as compared with TXA₂ to result in a significantly decreased PGI₂/TXA₂ ratio in the lung tissue.

We observed that administration of sevoflurane...
down-regulated the expression levels of COX-2 and 5-LOX as well as the pulmonary contents of LTB₄, TXA₂ and PGI₂, and alleviated lung injuries induced by OLV. These results indicate that the protective effects of sevoflurane against OLV-induced ALI are probably mediated by inhibiting COX-2 and 5-LOX pathways. However, the specific molecular mechanisms of such effects remain to be further studied.

Another finding in this study was that sevoflurane can increase the lung PGI₂/TXA₂ ratio in OLV induced ALI. This effect might be attributed to the inhibition of the vicious cycle of TXA₂ generation by sevoflurane through down-regulating 5-LOX expression, and to the greater sensitivity of COX-1 to sevoflurane than COX-2.
This hypothesis is supported by evidences from other studies. First, studies in vivo and in vitro showed that sevoflurane has strong anti-aggregatory effects by inhibiting TXA₂ formation and suppressing cyclooxygenase activity, even at a subanesthetic concentration of 0.5% \(^{13\text{-}14}\). Wacker and colleagues showed that AA-induced platelet aggregation could be inhibited by inhalation of low-concentration sevoflurane (less than 1% end-tidal) \(^{15}\). As a potent stimulator of platelet aggregation, TXA₂ is the predominant product of COX in platelets which contain only COX₁, but not COX₂ \(^{4}\).

Such studies indicated that the anti-aggregatory effects of sevoflurane were mediated by suppressing COX₁, even at low concentrations. Secondly, the study of constant-flow perfused lungs of Japanese white rabbits showed that cyclooxygenase products did not mediate the inhibition of hypoxic pulmonary vasoconstriction (HPV) by 1.2 MAC (approximately 2%) sevoflurane \(^{16}\). The in vitro study of human feto-placental vasculature showed that at the concentrations of 2% and 8%, sevoflurane-mediated vasodilation was cyclooxygenase-independent \(^{17}\). Likewise, Heindl et al. found that sevoflurane at 2 MAC, but not at 1 MAC, could reduce stimulated endothelial PGI₂ production by about 50\% \(^{18}\). PGI₂ is a potent vasodilator generated mainly by COX₂.
从COX₂和5-LOX途径探讨七氟醚抗单肺通气致急性肺损伤的作用机制

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摘要：目的 从COX₂和S-LOX途径探讨七氟醚抗单肺通气致急性肺损伤作用机制。方法 18只健康日本大耳白兔随机分为假手术组(S组)、单肺通气组(O组)、单肺通气+七氟醚组(OS组)。每组6只。用Western blotting和定量RT-PCR分别检测肺组织环氧酶(2-COX₂)、S-LOX和mRNA表达水平。ELISA检测肺组织自细胞三烯B₃(LETB₃)、血栓素A₂(TXA₂)。结果与S组相比，O组和OS组动物肺组织COX₂、5-LOX蛋白和mRNA表达水平、TXA₂、PGI₂含量、肺W/D比值和肺组织学评分明显升高(P<0.05),而肺组织PGL₂/TXA₂比值明显降低(P<0.05)。与O组相比，OS组肺组织PGL₂/TXA₂、PGL₂/TXA₂,比值明显增高(P<0.05),而其它各项指标均明显降低(P>0.05)。结论 本研究首次证实单肺通气可使实验动物肺组织COX₂、5-LOX的蛋白和mRNA表达水平、减少肺组织PGL₂/TXA₂、TXA₂和PGI₂生成及调控PGL₂/TXA₂比值。关键词：单肺通气;七氟醚;环氧酶-2;S-脂氧化酶

REFERENCES