

Spyridon Fortis  
Peter M. Spieth  
Wei-Yang Lu  
Matteo Parotto  
Jack J. Haitzma  
Arthur S. Slutsky  
Nanshan Zhong  
C. David Mazer  
Haibo Zhang

## Effects of anesthetic regimes on inflammatory responses in a rat model of acute lung injury

Received: 28 June 2011  
Accepted: 17 May 2012  
Published online: 19 June 2012  
© Copyright jointly held by Springer and ESICM 2012

S. Fortis and P. M. Spieth contributed equally to the work.

S. Fortis · P. M. Spieth · M. Parotto ·  
J. J. Haitzma · A. S. Slutsky ·  
C. D. Mazer · H. Zhang (✉)  
Keenan Research Center, Li Ka Shing  
Knowledge Institute of St. Michael's  
Hospital, University of Toronto,  
Room 619 LKSKI, 30 Bond Street,  
Toronto, ON M5B 1W8, Canada  
e-mail: haibo.zhang@utoronto.ca;  
zhangh@smh.ca  
Tel.: +1-416-8646060  
Fax: +1-416-8645277

S. Fortis · P. M. Spieth · M. Parotto ·  
C. D. Mazer · H. Zhang  
Department of Anesthesia,  
University of Toronto,  
Toronto, ON, Canada

S. Fortis · P. M. Spieth · M. Parotto ·  
J. J. Haitzma · A. S. Slutsky ·  
C. D. Mazer · H. Zhang  
Interdepartmental Division of Critical Care  
Medicine, University of Toronto,  
Toronto, ON, Canada

S. Fortis · P. M. Spieth · M. Parotto ·  
C. D. Mazer · H. Zhang  
Department of Physiology,  
University of Toronto,  
Toronto, ON, Canada

P. M. Spieth  
Department of Anesthesiology and  
Intensive Care Therapy, University Hospital  
Dresden, Dresden, Germany

W.-Y. Lu  
Robarts Research Institute,  
University of Western Ontario,  
London, ON, Canada

A. S. Slutsky · N. Zhong · H. Zhang  
State Key Laboratory in Respiratory  
Diseases, Guangzhou Medical University,  
Guangzhou, China

**Abstract Purpose:** Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter through activation of GABA receptors. Volatile anesthetics activate type-A (GABA<sub>A</sub>) receptors resulting in inhibition of synaptic transmission. Lung epithelial cells have been recently found to express GABA<sub>A</sub> receptors that exert anti-inflammatory properties. We hypothesized that the volatile anesthetic sevoflurane (SEVO) attenuates lung inflammation through activation of lung epithelial GABA<sub>A</sub> receptors. **Methods:** Sprague–Dawley rats were anesthetized with SEVO or ketamine/xylazine (KX). Acute lung inflammation was induced by intratracheal instillation of endotoxin, followed by mechanical ventilation for 4 h at a tidal volume of 15 mL/kg without positive end-expiratory

pressure (two-hit lung injury model). To examine the specific effects of GABA, healthy human lung epithelial cells (BEAS-2B) were challenged with endotoxin in the presence and absence of GABA with and without addition of the GABA<sub>A</sub> receptor antagonist picrotoxin.

**Results:** Anesthesia with SEVO improved oxygenation and reduced pulmonary cytokine responses compared to KX. This phenomenon was associated with increased expression of the  $\pi$  subunit of GABA<sub>A</sub> receptors and glutamic acid decarboxylase (GAD). The endotoxin-induced cytokine release from BEAS-2B cells was attenuated by the treatment with GABA, which was reversed by the administration of picrotoxin. **Conclusion:** Anesthesia with SEVO suppresses pulmonary inflammation and thus protects the lung from the two-hit injury. The anti-inflammatory effect of SEVO is likely due to activation of pulmonary GABA<sub>A</sub> signaling pathways.

**Keywords** Volatile anesthetics · Mechanical ventilation · Inflammation

## Introduction

Acute lung injury (ALI) is a major challenge in critical illness that is associated with high mortality [1]. The majority of patients suffering from ALI require mechanical ventilation for life support [2, 3]. However, mechanical ventilation per se bears the risk of inducing and worsening pulmonary dysfunction described as ventilator-induced lung injury (VILI) [4]. An important approach to manage patients with ALI is to reduce pulmonary and systemic inflammatory responses that may have played an important role in inducing multiple distal organ dysfunction [1, 2].

Low tidal volume ventilation has been considered as a protective ventilator strategy [2], but it may not suit all patients with respiratory failure [5]. Volatile anesthetics have been recently shown to exert anti-inflammatory effects in several experimental and clinical settings [6–8], which may be beneficial in the context of VILI. The use of sevoflurane (SEVO) can increase expression of I $\kappa$ B while decreasing NF- $\kappa$ B nuclear translocation following challenge with tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) in human monocytes [8]. The administration of SEVO improved gas exchange and attenuated lung injury after endotracheal installation of endotoxin in rats [6]. Anesthesia with SEVO has been shown to decrease the production of inflammatory cytokines including TNF- $\alpha$ , interleukin-6 (IL-6), and IL-8 in lung lavage fluids and reduced post-operative adverse events in patients under thoracic surgery [7]. However, the mechanisms by which SEVO exerts the anti-inflammatory effects remain unclear.

It is known that volatile anesthetics such as SEVO activate type-A gamma-aminobutyric acid (GABA<sub>A</sub>) receptors resulting in inhibition of synaptic transmission in neurons [9, 10]. GABA synthesized from glutamate by decarboxylation via the enzymatic activity of glutamic acid decarboxylase (GAD) produces fast synaptic inhibition in neurons through activating GABA<sub>A</sub> receptors, a GABA-gated anion channel. In addition to its conventional role in synaptic transmission, GABA<sub>A</sub> receptors also exert novel anti-inflammatory properties in the central nervous system [11] and in peripheral immune cells [12].

It is interesting that recent studies have demonstrated that GABA<sub>A</sub> receptors are also expressed in lung airway and alveolar epithelial cells [13–16]. However, their local role in response to inhalation of SEVO remains to be investigated in the context of ALI. We thus hypothesized that anesthesia with SEVO attenuates pulmonary inflammatory response through activation of GABA<sub>A</sub> receptors in lung epithelial cells. To test this hypothesis we examined the effects of SEVO on the expression of GABA<sub>A</sub> receptors and cytokine responses in a rat model of ALI, and the specific effects of GABA on cytokine responses in vitro in human lung epithelial cells.

## Materials and methods

### Anesthesia and mechanical ventilation

The study was approved by the institutional Animal Care Committee (ACC948) of St. Michael's Hospital. Thirty-four Sprague–Dawley rats (250–350 g) were randomly assigned to receive anesthesia with either ketamine + xylazine (KX,  $n = 17$ ) or sevoflurane (SEVO,  $n = 17$ ). In the KX group, anesthesia was induced using ketamine at 80 mg/kg and xylazine at 8 mg/kg intraperitoneally, followed by continuous intravenous (i.v.) infusion of ketamine at 15 mg/kg/h and xylazine at 3 mg/kg/h. In the SEVO group, anesthesia was induced by 3.5 vol % SEVO in a closed chamber and maintained with 2.6 vol %. These doses of anesthetics were chosen on the basis of pilot experiments targeting similar mean arterial blood pressure (MAP) and comparable depth of anesthesia defined by no reaction to toe pinches prior to neuromuscular blockade. Tracheotomy was performed followed by intratracheal intubation with a 14G angiocatheter. Neuromuscular blockade was achieved by i.v. infusion of pancuronium at 0.5 mg/kg/h.

The following initial settings were applied using a rodent ventilator (Harvard 683): inspired oxygen fraction (F<sub>I</sub>O<sub>2</sub>) of 0.40, positive end-expiratory pressure (PEEP) of 5 cmH<sub>2</sub>O, tidal volume (V<sub>T</sub>) of 6 mL/kg, and respiratory rate (RR) adjusted to keep arterial partial pressure of CO<sub>2</sub> (PaCO<sub>2</sub>) between 35 and 45 mmHg. The right carotid artery was cannulated (0.58 mm ID polyethylene tube) for continuous blood pressure monitoring. Intravenous infusion of lactated Ringer's solution at 3 mL/kg/h was administered through the tail vein in all animals. Rectal temperature was maintained at 37 ± 1 °C using a heating blanket.

### Two-hit model of ALI

All animals received intratracheal instillation of endotoxin at 5 mg/kg (LPS, *Escherichia coli* serotype 055:B5, Sigma-Aldrich). Thirty minutes later, mechanical ventilation was switched to high V<sub>T</sub> at 15 mL/kg and PEEP = 0 cmH<sub>2</sub>O for 4 h.

### Measurements

Mean arterial blood pressure was monitored in a real-time fashion. Arterial blood gases (PaO<sub>2</sub>, PaCO<sub>2</sub>) were recorded hourly throughout the experiments.

Bronchoalveolar lavage (BAL) was performed in the left lung by using cold normal saline upon completion of the experiments. The BAL fluid was centrifuged at 4 °C and 1,200×g for 10 min and the supernatant was stored at –80 °C for further analysis.

Lung wet/dry ratio (WD ratio) was obtained after excision of the right lung and drying at 60 °C for 72 h.

The concentrations of glucose and lactate in blood were measured (Radiometer ABL 700 blood gas analyzer) at the end of the study.

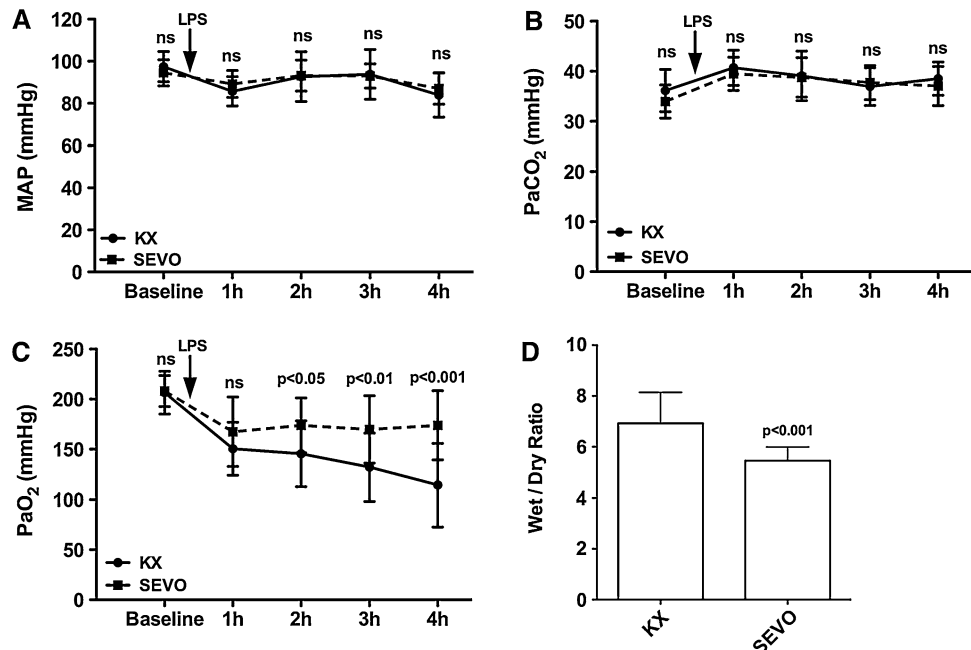
Levels of intercellular adhesion molecule 1 (ICAM-1) and multiple cytokines including interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-2, IL-4, IL-10, IL-12, keratinocyte chemoattractant (KC), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 alpha (MIP-1 $\alpha$ ), and tumor necrosis factor-alpha (TNF- $\alpha$ ) were measured using species-specific multiplex cytokine assays (Bio-Rad) as previously described [17].

Basal expression of GAD (GAD65/67 antibody, Abcam, Inc., Cambridge, MA) and GABA<sub>A</sub> receptors (GABA<sub>A</sub> receptor alpha 2 antibody, Abcam, Inc.) was detected by immunohistochemistry in rats killed by cervical dislocation without undergoing the experimental protocol to serve as healthy controls. The methods have been previously described by the authors [15]. GAD expression was also assessed by Western blot (GAD65/67 antibody, Abcam, Inc.) analysis as previously described [15].

#### Cell culture and treatment

Human bronchial epithelial cells (BEAS-2B, ATTC) were cultured in full confluence and incubated with GABA (100 nM) alone or GABA + picrotoxin, a GABA<sub>A</sub> receptor antagonist (PTX, 50 nM) for 10 min, followed by stimulation with LPS at 100 ng/mL (*Escherichia coli* serotype 055:B5, Sigma-Aldrich) for 4 h. This dose of LPS has been previously used for induction of inflammatory responses [18].

**Fig. 1** **a** Mean arterial blood pressure (MAP), **b** arterial partial pressure of carbon dioxide (PaCO<sub>2</sub>), and **c** arterial partial pressure of oxygen (PaO<sub>2</sub>) in animals anesthetized with ketamine/xylazine (KX) or sevoflurane (SEVO) over time. **d** Lung wet/dry weight ratio was determined at the end of 4-h mechanical ventilation started 30 min after LPS administration. *N* = 17 per group



#### Statistical analysis

Data are presented as mean and standard deviation. Student's *t* test for unpaired samples was used to compare differences among groups at time zero and for post-mortem analysis. Repeated measurements over time were analyzed with two-way analysis of variance for repeated measurements and adjusted with the Bonferroni post test. Statistical significance was considered if  $p < 0.05$ .

## Results

Body weight was similar in all animals. The amount of fluid infused was not different in both groups ( $11.3 \pm 0.7$  mL in SEVO group vs.  $12.5 \pm 1.4$  mL in KX group). Although the glucose level was slightly higher in the SEVO group than in the KX group ( $8.59 \pm 0.57$  mmol/L vs.  $7.83 \pm 0.18$  mmol/L) the difference did not reach statistical significance. However, a lower level of lactate in blood was observed in the SEVO group than the KX group ( $2.26 \pm 0.23$  mmol/L vs.  $3.35 \pm 0.45$  mmol/L,  $p < 0.05$ ).

#### Hemodynamics and gas exchange

Mean arterial blood pressure and PaCO<sub>2</sub> showed a similar time course in all animals (Fig. 1a, b respectively). Partial pressure of oxygen decreased significantly starting 2 h after high  $V_T$  ventilation in the KX group as compared to the SEVO group (Fig. 1c). The decreased level of PaO<sub>2</sub>

was associated with an increase in lung wet/dry ratio suggesting greater lung permeability in the KX group (Fig. 1d).

### Pulmonary inflammatory response

The total leukocyte count in BAL fluid was significantly lower in the SEVO group compared to the KX group ( $0.85 \pm 0.04 \times 10^6$  vs.  $1.03 \pm 0.05 \times 10^6$ ,  $p < 0.05$ ). Although there was a trend of decrease in the percentage of polymorphonuclear cells over the total leukocytes ( $55 \pm 5$  vs.  $69 \pm 6$  %), the difference did not reach statistical significance. The cytokine profile in BAL fluid showed lower levels of TNF- $\alpha$ , MIP-1 $\alpha$ , IL-1 $\beta$ , KC, MCP-1, and ICAM-1 under anesthesia with SEVO compared to that under anesthesia with KX (Fig. 2).

### Immunohistochemistry

Immunohistochemistry showed a basal expression of GAD and GABA<sub>A</sub>R $\alpha$  unit colocalized with lung epithelium stained with surfactant protein C of healthy rats (Fig. 3a). Western blot assay showed a higher protein level of GAD in the SEVO group than in the KX group (Fig. 3b).

### In vitro BEAS-2B cell experiments

To investigate the specific effects of GABA in lung epithelial cells, human BEAS-2B cells were stimulated with LPS followed by treatment with GABA, PTX, or GABA + PTX. We first confirmed that the dose of PTX used did not induce cytotoxicity as reflected by a constant LDH level before and after PTX administration [19]; thus, cell viability was not affected (data not shown). Interleukin-1 $\beta$  was significantly reduced with GABA and GABA + PTX compared to control and PTX alone group (Fig. 4a). Interleukin-2 was significantly decreased with GABA and PTX compared to GABA + PTX (Fig. 4b). The concentrations of IL-8 and IL-10 decreased significantly with GABA, which was reversed by administration of PTX (Fig. 4c, d respectively).

## Discussion

We demonstrate that anesthesia with SEVO improves oxygenation and reduced pulmonary cytokine responses associated with increased expression of GABA<sub>A</sub> as compared to anesthesia with KX. These results suggest that the activation of GABA<sub>A</sub> receptors may play a role in the lung-protective effect seen with SEVO.

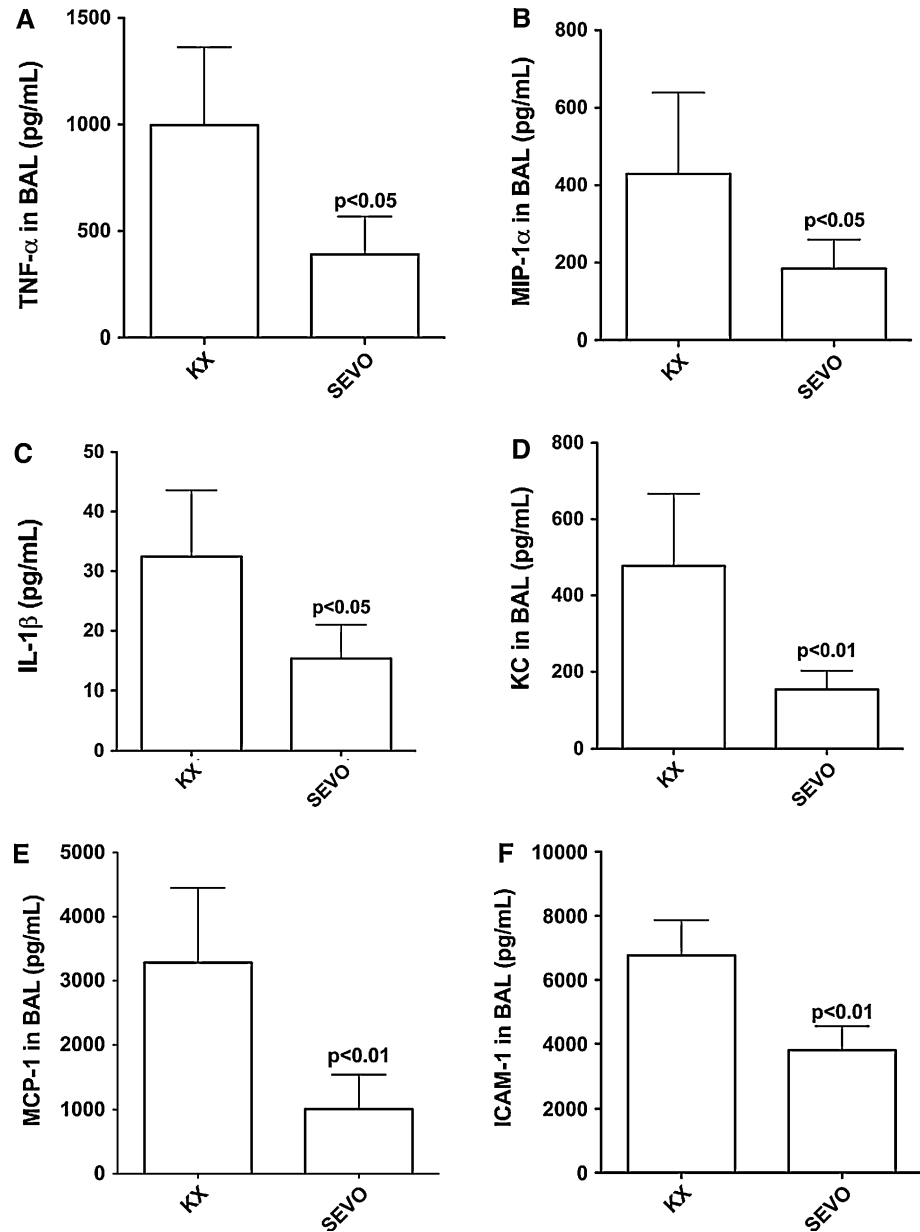
An established two-hit model was chosen for its clinical relevance in that primary lung inflammation/

injury was followed by mechanical ventilation as a treatment [6]. Since the use of either ketamine or xylazine alone is not recommended, a regime of the combination is commonly used for animal anesthesia [20]. We examined two regimes of anesthesia by choosing SEVO and KX in the present study. To avoid possible interference and/or crossover effects between the SEVO and KX, the same anesthetic regime was used for both induction and maintenance. Because the two anesthetic regimes function through distinct mechanisms of action, this would help us understand the possible signaling pathways responsible for their effects. SEVO acts mainly via agonistic effects on GABA<sub>A</sub> receptors. Ketamine interacts mainly with the NMDA-subtype glutamate receptor, but at high, fully anesthetic level doses, it binds to opioid  $\mu$  receptors and sigma receptors [21–23].

In comparison with SEVO, ketamine has minor effects by selectively acting on  $\alpha 6$  and  $\delta$  subunits of GABA receptors [22]. The majority of literature suggested that ketamine exerts antagonistic action at the GABA<sub>A</sub> receptor complex [24–26]. In particular, ketamine has been reported to decrease the GAD67 isoform [27]. Our results are in agreement with these previous studies [25–27]. It is noted that subanesthetic doses of ketamine reportedly inhibit tonic convulsions induced by the GABA<sub>A</sub> receptor antagonist bicuculline, and the latter antagonized ketamine anesthesia [28]. However, the suggested agonistic effects of ketamine on GABA<sub>A</sub> are yet to be examined specifically under in vitro and in vivo conditions. Xylazine is a clonidine analogue acting on presynaptic and postsynaptic receptors of the central and peripheral nervous systems as an  $\alpha_2$ -adrenergic agonist that is used primarily for sedation, anesthesia, analgesia, and muscle relaxation in animal models [29]. Similar drugs such as clonidine and dexmedetomidine are increasingly used in clinical anesthesia and critical care [30]. The different pharmacological actions of the anesthetics used in this study may explain their distinct anti-inflammatory properties. In addition, paralysis was applied by administration of a given dose of pancuronium in all animals to achieve animal–ventilator synchrony in order to minimize signal to noise ratio and to enhance reproducibility of the experiments. It is therefore evident that the paralysis was part of the anesthesia regimes used in the present study.

Various inflammatory mediators including the cytokines TNF- $\alpha$  and IL-1 $\beta$ , chemokines, and ICAM-1 have been shown to act as effector molecules in the disruption of the epithelial and endothelial barrier during mechanical ventilation [31, 32]. We observe that the administration of SEVO attenuated the production of the inflammatory mediators in the lung and thus reduced lung permeability. Our results are in agreement with other studies showing anti-inflammatory effects of volatile anesthetics in a variety of in vivo and in vitro models. In an ischemia–reperfusion model of acute kidney injury, Lee et al. [33]

**Fig. 2** Cytokine and ICAM-1 responses in animals anesthetized with ketamine/xylazine (KX) or sevoflurane (SEVO). Bronchoalveolar lavage (BAL) fluid was obtained at the end of 4-h mechanical ventilation started 30 min after LPS intratracheal administration. **a** Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), **b** macrophage inflammatory protein-1  $\alpha$  (MIP-1 $\alpha$ ), **c** interleukin-1  $\beta$  (IL-1 $\beta$ ), **d** keratinocyte growth factor (KC), **e** monocyte chemoattractant protein-1 (MCP-1), and **f** intracellular adhesion molecule-1 (ICAM-1).  $N = 17$  per group

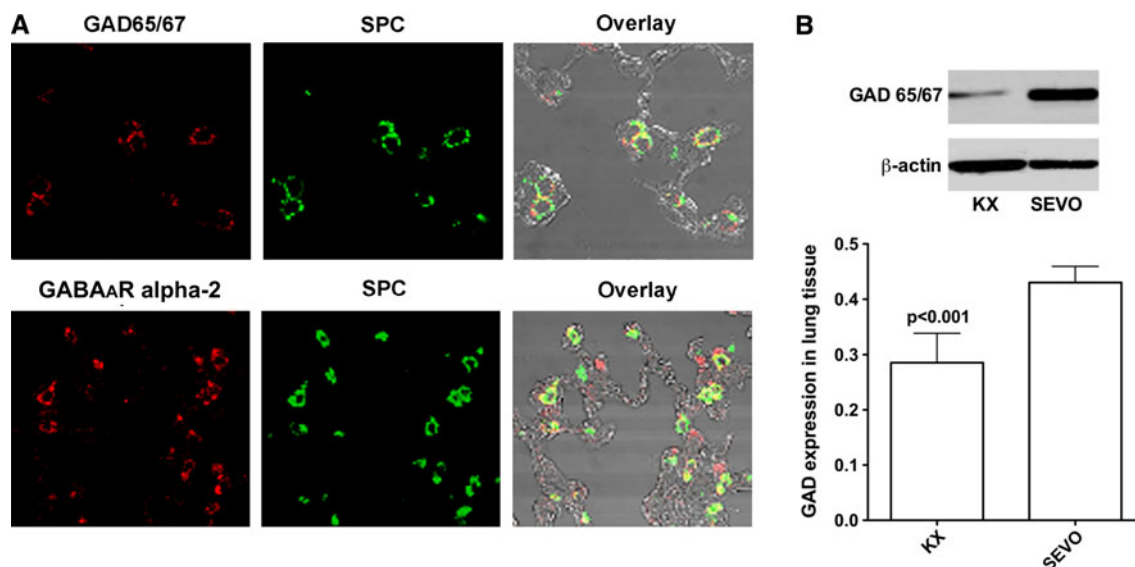


reported direct anti-inflammatory and antinecrotic effects of SEVO by activation of prosurvival kinases and increase in de novo synthesis of heat shock protein 70. Boost et al. [8] demonstrated that isoflurane, acting also on GABA<sub>A</sub> receptors [34], attenuates the release of IL-8 and heme oxygenase 1 in human monocytic THP-1 cells in vitro through a mechanism by which the volatile anesthetic stabilizes the NF- $\kappa$ B inhibitory pathway.

Interleukin-10 was originally discovered as the cytokine synthesis inhibiting factor on the basis of its biological activity. It downregulates the expression of pro-inflammatory cytokines such as IL-1 $\beta$ , MHC class II antigens, and co-stimulatory molecules by upregulation of itself. In the present study, the administration of GABA

attenuated the LPS-induced production of IL-1 $\beta$  and IL-10 in BEAS-2B cells. This phenomenon is consistent with previous reports showing that concentrations of IL-1 $\beta$  and IL-10 increased in response to LPS challenge to counterbalance the pro- and anti-inflammatory responses [35, 36].

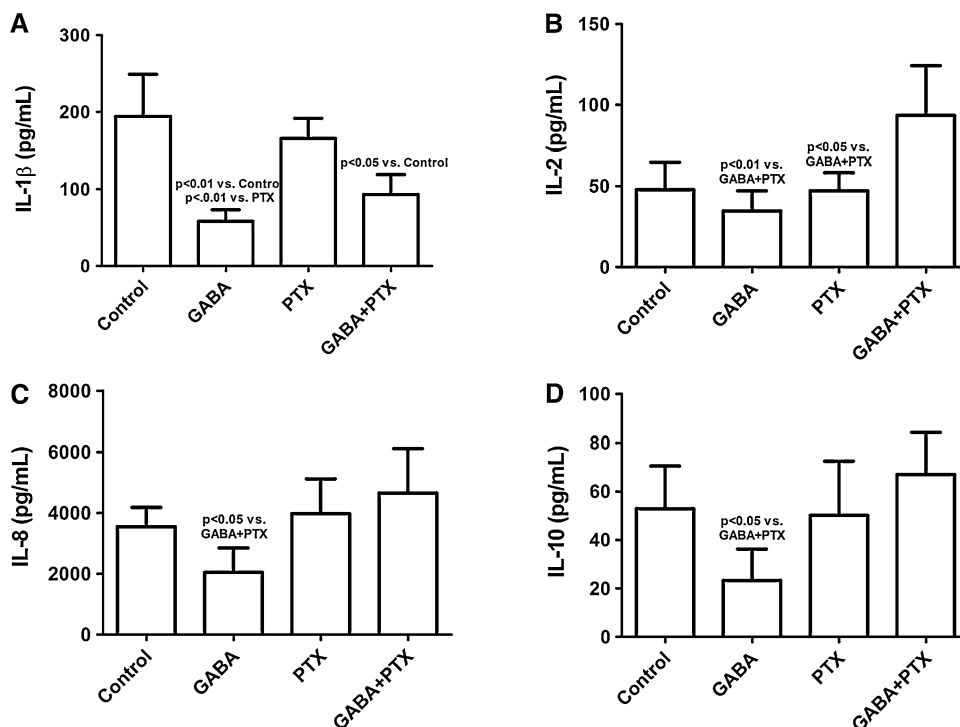
A recent study by Faller and colleagues [37] reported that the administration of the volatile anesthetic isoflurane resulted in a reduction in lung damage, inflammation, and stress protein expression in a mouse model of VILI using a tidal volume of 12 mL/kg for 6 h. Our data are consistent with their observation despite that we use a two-hit model of ALI followed mechanical ventilation under the anesthesia with SEVO. Taken together, our results and the



**Fig. 3** Lung expression of GAD and GABA<sub>A</sub>R receptor. **a** Immunohistochemistry staining for the epithelial marker surfactant protein C (SPC; *green*), and GAD (*red*), GABA<sub>A</sub>R (*red*) and their overlay (*orange*) in healthy rat lungs. **b** Western blot for detection

of GAD in animals anesthetized with KX or SEVO at the end of 4-h mechanical ventilation started 30 min after LPS intratracheal administration. Average expression of GAD over  $\beta$ -actin was from five experiments

**Fig. 4** Cytokine responses in BEAS-2B cells. Concentration of **a** interleukin-1 $\beta$ , **b** interleukin-2, **c** interleukin-8, and **d** interleukin-10 measured in cell culture supernatants of human bronchial epithelial cells (BEAS-2B) stimulated with LPS (control) followed by incubation with gamma-aminobutyric acid (GABA), picrotoxin (PTX), or GABA + PTX for 8 h.  $N = 5$  experiments per group



previous study [37] support the concept that volatile anesthetics exert anti-inflammatory effects protecting the lung from injury.

Volatile anesthetics produce anesthetic action primarily by binding to GABA<sub>A</sub> receptors in neurons of the central nervous system (CNS) [15]. Specifically, SEVO activates GABA<sub>A</sub> receptors in the CNS resulting in an

inhibition of neuronal activities [9, 15]. We have previously discovered the existence of GAD and GABA receptors in human airway epithelial cells [15], and other investigators identified GABA receptors in alveolar epithelial type II cells [14]. In an ongoing study, we observed that volatile anesthetics including SEVO also enhance GABA<sub>A</sub> receptor-mediated anion current in pulmonary



epithelial cells. In the present study, we further confirmed the presence of GAD and GABA<sub>A</sub> receptors both in rat airway and alveolar epithelial cells. We also demonstrate that anesthesia with SEVO results in an increase in expression of GAD and GABA<sub>A</sub> receptors in the lung compared with KX. We speculate that an increase in GAD expression after SEVO administration may be due to a positive feedback mechanism as a result of the binding between SEVO and GABA<sub>A</sub> receptors.

The concentrations of cytokines were lower and the GAD expression was higher in the SEVO group than in the KX group under in vivo conditions. These observations suggest that SEVO exerts anti-inflammatory properties partially by upregulation of GABA receptors. This concept was further supported by the in vitro lung epithelial cell studies where LPS-induced cytokine responses were attenuated in the presence of GABA, which was reversed by the administration of picrotoxin. Furthermore, it has been reported that an increase in surface levels of GABA<sub>A</sub> receptors requires the activity of phosphoinositide 3-kinase (PI3K) and protein kinase C [38]. This is in accord with a recent study reporting that a mechanism by which isoflurane reduced VILI was through increasing phosphorylation of Akt protein, since the inhibition of phosphoinositide 3-kinase/Akt signaling prior to mechanical ventilation completely reversed the lung-protective effects of isoflurane in mice [37].

It is noteworthy that anesthesia with SEVO or KX may result in hyperglycemia in rats [39, 40]. Clinical and experimental data suggest that hyperglycemia is protective against the development of ALI/ARDS [41]. We observe that the blood glucose level was higher in the SEVO group than in the KX group although the difference did not reach statistical significance. However, whether a different effect on glycemia between SEVO

and KX might provide an additional pathway by which SEVO exerts a protective effect remains to be further investigated.

There are several limitations in the study. (1) The experimental double-hit model used may not reflect the complex clinical scenario seen in patients with ALI/ARDS. (2) The involvement of GABA<sub>A</sub> receptors was the focus of the study; whether other mechanisms are also responsible for the protective effects of SEVO remains to be elucidated. (3) As the study design compares the modulation of pulmonary inflammatory responses during mechanical ventilation by two different anesthesia regimens, it is impossible to conclude whether the difference is due to the protective effects of one or the detrimental effects of the other single agent. (4) We focused the investigation on SEVO in the present study; further studies using other volatile (e.g. desflurane or isoflurane) or intravenous (i.e. propofol or midazolam) anesthetics are warranted.

In summary, we demonstrate that anesthesia with sevoflurane can improve oxygenation and reduce pulmonary cytokine responses as compared to ketamine/xylazine. The protective effects of sevoflurane appear to be associated with its agonistic effects at GABA<sub>A</sub> receptors.

**Acknowledgments** The authors are indebted to Julie Khang, BSc for technical assistance. The study was supported by Canadian Institutes of Health Research (CIHR) grants to WYL, Arthur S. Slutsky, and Haibo Zhang, Ontario Thoracic Society (OTS) Grant-in-Aid and McLaughlin Foundation to Haibo Zhang. Spyridon Fortis was supported by the Alexander S. Onassis Public Benefit Foundation. Peter M. Spieth was supported by the German Research Foundation (DFG SP 1222/3-1). Peter M. Spieth is a recipient of the University of Toronto Eli Lilly Critical Care Fellowship Award.

## References

- Phua J, Badia JR, Adhikari NK, Friedrich JO, Fowler RA, Singh JM, Scales DC, Stather DR, Li A, Jones A, Gattas DJ, Hallett D, Tomlinson G, Stewart TE, Ferguson ND (2009) Has mortality from acute respiratory distress syndrome decreased over time? A systematic review. *Am J Respir Crit Care Med* 179:220–227
- The Acute Respiratory Distress Syndrome Network (2000) Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med* 342:1301–1308
- Brun-Buisson C, Minelli C, Bertolini G, Brazzi L, Pimentel J, Lewandowski K, Bion J, Romand J, Villar J, Thorsteinsson A, Damas P, Armaganidis A, Lemaire F, Alive Study Group (2004) Epidemiology and outcome of acute lung injury in European intensive care units. Results from the ALIVE study. *Intensive Care Med* 30:51–61
- Tremblay L, Valenza F, Ribeiro SP, Li J, Slutsky AS (1997) Injurious ventilatory strategies increase cytokines and c-fos mRNA expression in an isolated rat lung model 4. *J Clin Invest* 99:944–952
- Malhotra A (2007) Low-tidal-volume ventilation in the acute respiratory distress syndrome. *N Engl J Med* 357:1113–1120
- Voigtsberger S, Lachmann RA, Leutert AC, Schlapfer M, Booy C, Reyes L, Urner M, Schild J, Schimmer RC, Beck-Schimmer B (2009) Sevoflurane ameliorates gas exchange and attenuates lung damage in experimental lipopolysaccharide-induced lung injury. *Anesthesiology* 111:1238–1248
- De Conno E, Steurer MP, Wittlinger M, Zalunardo MP, Weder W, Schneiter D, Schimmer RC, Klaghofer R, Neff TA, Schmid ER, Spahn DR, Z'Graggen BR, Urner M, Beck-Schimmer B (2009) Anesthetic-induced improvement of the inflammatory response to one-lung ventilation. *Anesthesiology* 110:1316–1326

8. Boost KA, Leipold T, Scheiermann P, Hoegl S, Sadik CD, Hofstetter C, Zwissler B (2009) Sevoflurane and isoflurane decrease TNF-alpha-induced gene expression in human monocytic THP-1 cells: potential role of intracellular I kappa B alpha regulation. *Int J Mol Med* 23:665–671
9. Hirota K, Roth SH (1997) Sevoflurane modulates both GABA<sub>A</sub> and GABA<sub>B</sub> receptors in area CA1 of rat hippocampus. *Br J Anaesth* 78:60–65
10. Sebel LE, Richardson JE, Singh SP, Bell SV, Jenkins A (2006) Additive effects of sevoflurane and propofol on gamma-aminobutyric acid receptor function. *Anesthesiology* 104:1176–1183
11. Bhat R, Axtell R, Mitra A, Miranda M, Lock C, Tsien RW, Steinman L (2010) Inhibitory role for GABA in autoimmune inflammation. *Proc Natl Acad Sci USA* 107:2580–2585
12. Duthey B, Hubner A, Diehl S, Boehncke S, Pfeffer J, Boehncke WH (2010) Anti-inflammatory effects of the GABA<sub>B</sub> receptor agonist baclofen in allergic contact dermatitis. *Exp Dermatol* 19:661–666
13. Jin N, Kolliputi N, Gou D, Weng T, Liu L (2006) A novel function of ionotropic gamma-aminobutyric acid receptors involving alveolar fluid homeostasis. *J Biol Chem* 281:36012–36020
14. Jin N, Narasaraju T, Kolliputi N, Chen J, Liu L (2005) Differential expression of GABA<sub>A</sub> receptor pi subunit in cultured rat alveolar epithelial cells. *Cell Tissue Res* 321:173–183
15. Xiang YY, Wang S, Liu M, Hirota JA, Li J, Ju W, Fan Y, Kelly MM, Ye B, Orser B, O'Byrne PM, Inman MD, Yang X, Lu WY (2007) A GABAergic system in airway epithelium is essential for mucus overproduction in asthma. *Nat Med* 13:862–867
16. Mizuta K, Osawa Y, Mizuta F, Xu D, Emala CW (2008) Functional expression of GABA<sub>B</sub> receptors in airway epithelium. *Am J Respir Cell Mol Biol* 39:296–304
17. Voglis S, Quinn K, Tullis E, Liu M, Henriques M, Zubrinich C, Penuelas O, Chan H, Silverman F, Cherepanov V, Orzech N, Khine AA, Cantin A, Slutsky AS, Downey GP, Zhang H (2009) Human neutrophil peptides and phagocytic deficiency in bronchiectatic lungs. *Am J Respir Crit Care Med* 180:159–166
18. Zhang H, Kim YK, Govindarajan A, Baba A, Binnie M, Marco Ranieri V, Liu M, Slutsky AS (1999) Effect of adrenoceptors on endotoxin-induced cytokines and lipid peroxidation in lung explants. *Am J Respir Crit Care Med* 160:1703–1710
19. Racher AJ, Looby D, Griffiths JB (1990) Use of lactate dehydrogenase release to assess changes in culture viability. *Cytotechnology* 3:301–307
20. Green CJ, Knight J, Precious S, Simpkin S (1981) Ketamine alone and combined with diazepam or xylazine in laboratory animals: a 10 year experience. *Lab Anim* 15:163–170
21. Narita M, Yoshizawa K, Aoki K, Takagi M, Miyatake M, Suzuki T (2001) A putative sigma1 receptor antagonist NE-100 attenuates the discriminative stimulus effects of ketamine in rats. *Addict Biol* 6:373–376
22. Hevers W, Hadley SH, Luddens H, Amin J (2008) Ketamine, but not phencyclidine, selectively modulates cerebellar GABA<sub>A</sub> receptors containing alpha6 and delta subunits. *J Neurosci* 28:5383–5393
23. Hirota K, Sikand KS, Lambert DG (1999) Interaction of ketamine with mu2 opioid receptors in SH-SY5Y human neuroblastoma cells. *J Anesth* 13:107–109
24. Kounenis G, Koutsoviti-Papadopoulou M, Elezoglou V (1995) Ketamine may modify intestinal motility by acting at GABA<sub>A</sub>-receptor complex; an in vitro study on the guinea pig intestine. *Pharmacol Res* 31:337–340
25. Vutskits L, Gascon E, Potter G, Tassonyi E, Kiss JZ (2007) Low concentrations of ketamine initiate dendritic atrophy of differentiated GABAergic neurons in culture. *Toxicology* 234:216–226
26. Sonner JM, Zhang Y, Stabernack C, Abaigar W, Xing Y, Laster MJ (2003) GABA<sub>A</sub> receptor blockade antagonizes the immobilizing action of propofol but not ketamine or isoflurane in a dose-related manner. *Anesth Analg* 96:706–712
27. Zhang Y, Behrens MM, Lisman JE (2008) Prolonged exposure to NMDAR antagonist suppresses inhibitory synaptic transmission in prefrontal cortex. *J Neurophysiol* 100:959–965
28. Irifune M, Sato T, Kamata Y, Nishikawa T, Dohi T, Kawahara M (2000) Evidence for GABA<sub>A</sub> receptor agonistic properties of ketamine: convulsive and anaesthetic behavioural models in mice. *Anesth Analg* 91:230–236
29. Garcia-Villar R, Toutain PL, Alvinerie M, Ruckebusch Y (1981) The pharmacokinetics of xylazine hydrochloride: an interspecific study. *J Vet Pharmacol Ther* 4:87–92
30. Riker RR, Shehabi Y, Bokesch PM, Ceraso D, Wisemandle W, Koura F, Whitten P, Margolis BD, Byrne DW, Ely EW, Rocha MG (2009) Dexmedetomidine vs midazolam for sedation of critically ill patients: a randomized trial. *JAMA* 301:489–499
31. Bruewer M, Luegering A, Kucharzik T, Parkos CA, Madara JL, Hopkins AM, Nusrat A (2003) Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. *J Immunol* 171:6164–6172
32. Choi H, Fleming NW, Serikov VB (2007) Contact activation via ICAM-1 induces changes in airway epithelial permeability in vitro. *Immunol Invest* 36:59–72
33. Lee HT, Kim M, Jan M, Emala CW (2006) Anti-inflammatory and antineurotic effects of the volatile anaesthetic sevoflurane in kidney proximal tubule cells. *Am J Physiol Renal Physiol* 291:F67–F78
34. Jia F, Yue M, Chandra D, Homanics GE, Goldstein PA, Harrison NL (2008) Isoflurane is a potent modulator of extrasynaptic GABA<sub>A</sub> receptors in the thalamus. *J Pharmacol Exp Ther* 324:1127–1135
35. Erickson MA, Banks WA (2011) Cytokine and chemokine responses in serum and brain after single and repeated injections of lipopolysaccharide: multiplex quantification with path analysis. *Brain Behav Immun* 25:1637–1648
36. Ward JL, Harting MT, Cox CS Jr, Mercer DW (2011) Effects of ketamine on endotoxin and traumatic brain injury induced cytokine production in the rat. *J Trauma* 70:1471–1479
37. Faller S, Strosing KM, Ryter SW, Buerkle H, Loop T, Schmidt R, Hoetzel A (2012) The volatile anaesthetic isoflurane prevents ventilator-induced lung injury via phosphoinositide 3-kinase/Akt signaling in mice. *Anesth Analg* 114:747–756
38. Porcher C, Hatchett C, Longbottom RE, McAinch K, Sihra TS, Moss SJ, Thomson AM, Jovanovic JN (2011) Positive feedback regulation between gamma-aminobutyric acid type A (GABA<sub>A</sub>) receptor signaling and brain-derived neurotrophic factor (BDNF) release in developing neurons. *J Biol Chem* 286:21667–21677
39. Kaufman DA (2009) Time-dependent behavioral recovery after sepsis in rats. *Intensive Care Med* 35:576; author reply 577
40. Kitamura T, Ogawa M, Kawamura G, Sato K, Yamada Y (2009) The effects of sevoflurane and propofol on glucose metabolism under aerobic conditions in fed rats. *Anesth Analg* 109:1479–1485
41. Honiden S, Gong MN (2009) Diabetes, insulin, and development of acute lung injury. *Crit Care Med* 37:2455–2464