

Distinct Effects of Sevoflurane and Propofol on Vascular Permeability *In Vitro* and in a Mouse Model

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Endothelial cells on the inner surface of blood vessels play an important role in vascular permeability
- The question of how general anesthetics affect endothelial vascular permeability remains incompletely explored

What This Article Tells Us That Is New

- *In vitro*, sevoflurane dose-dependently increases permeability of both human umbilical vein and mouse pulmonary endothelial cells, while propofol does not have such effects
- The effects of sevoflurane on endothelial permeability are mediated *via* the hypoxia-inducible factor 1 α /vascular endothelial growth factor pathway
- In mice, sevoflurane but not propofol increases pulmonary vascular permeability

Anesthesia is generally considered safe. However, complications associated with general anesthesia are also well known. Two main classes of general anesthetics, inhaled

ABSTRACT

Background: General anesthetics may substantially influence endothelium function, potentially affecting outcomes of surgical patients, but their effects are unclear. Here, the authors studied a commonly used inhaled anesthetic, sevoflurane, and an intravenous anesthetic, propofol, on vascular endothelial permeability using multiple *in vitro* assays and a mouse model.

Methods: Human umbilical vein endothelial cells and mouse pulmonary endothelial cells (MPECs) were used for *in vitro* models to test the effect of anesthetics on endothelial permeability. The effect of anesthetics on pulmonary vascular leakage was analyzed using AngioSense 750 (PerkinElmer, USA) fluorescent tracer and rhodamine-labeled 3-kD dextran in a mouse model. Downstream targets were identified using RNA sequencing and confirmed by quantitative real-time polymerase chain reaction and Western blot.

Results: Sevoflurane at clinically relevant concentrations disrupted the endothelial monolayer formed by human umbilical vein endothelial cells and MPECs in transwell permeability models. Sevoflurane, but not propofol, induced a 1.8-fold increase of AngioSense dye accumulation in mouse lung over control, indicating pulmonary vascular leakage in the sevoflurane group. RNA sequencing analysis, quantitative real-time polymerase chain reaction, and Western blot analysis revealed that sevoflurane induced the expression and activation of hypoxia-inducible factor 1 α (HIF-1 α) *in vitro* and *in vivo*. The activation of HIF-1 α led to the increased expression of its downstream vascular endothelial growth factor (VEGF). The knockdown of HIF-1 α restored the change of endothelial permeability and abolished the increase of VEGF induced by sevoflurane in MPECs.

Conclusions: The authors' results demonstrate that sevoflurane increased endothelial and pulmonary vascular permeability *via* HIF-1 α and VEGF. Propofol had no significant effect on the permeability of endothelium.

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(inhalational, volatile; mostly sevoflurane) and intravenous anesthetics (mainly propofol), are used to induce and maintain general anesthesia, but they have differential effects on many aspects of biologic processes. Under general anesthesia, the entire vasculature of the body is exposed to anesthetics. It has been shown that general anesthetics affect the vascular system.¹ Sevoflurane decreases systemic vascular resistance by suppressing endothelium-dependent vascular tone,² while propofol decreases systemic vascular resistance

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predominantly in an endothelium-independent manner.³ Endothelial cells, the inner surface of blood vessels, play the critical role in control of vascular permeability.⁴ The effect of anesthetics on this aspect of endothelial cells is unclear.

Vascular endothelial growth factor (VEGF) is one of the most important regulators in the functions of endothelial cells.⁵ VEGF increases vascular permeability by promoting the reorganization of endothelial cell junctions such as Zonula Occludens-1 and occludin and inducing the formation of fenestrations between endothelial cells.⁶ Increased vascular permeability can be a physiologic response to facilitate the passage of immune cells and molecules to sites of inflammation. However, prolonged elevation of vascular permeability contributes to many diseases, such as cancer metastasis and tissue edema.^{6,7}

In our previous study on the effects of general anesthetics on lung metastasis of breast cancer cells in mouse models, we found that sevoflurane led to significantly more pulmonary metastases than propofol after mastectomy.⁸ We also observed that an increase of VEGF in the lung was associated with sevoflurane but not propofol, which suggested that sevoflurane affected the functions of pulmonary vasculature. In addition to cancer cell metastasis, regulation of vascular permeability is associated with a broad spectrum of diseases^{9–13}; thus, the effects of general anesthetics might have significant and vast clinical relevance. Here, we test our hypothesis that sevoflurane and propofol differentially affect vascular permeability and tested our hypothesis with both *in vivo* and *in vitro* models.

Materials and Methods

Ethics Statement

All of the animals used in the experimental procedures were approved by the Institutional Animal Care and Use Committee at Stony Brook University (917821) and purchased from The Jackson Laboratory (USA). All the animal procedures were performed in accordance with Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines (Supplemental Digital Content table 1, <https://links.lww.com/ALN/D898>). All animals were housed in sterilized plastic cages under pathogen-free conditions (21° to 25°C; 12-h/12-h light/dark cycle). Food and water were offered *ad libitum*. All efforts were made to ameliorate the suffering of the animal during euthanasia using carbon dioxide overdose followed by cervical dislocation. GraphPad (GraphPad Software, USA) (randomly assign subjects to treatment groups, assign six subjects to each of three groups, repeat one time) was used to randomize the mice to different anesthetic treatments. All the mice received the treatments on the same day, and all the cages were in the same room. Administration of general anesthetics was not blinded to the performers because sevoflurane was inhaled, and propofol was injected intraperitoneally. However, AngioSense 750 (PerkinElmer, USA) *ex vivo* experiments and subsequent data

analyses were blinded to group allocations. After induction of anesthesia by either sevoflurane or propofol, the depth of anesthesia was verified by lack of response to firmly pinching the rear paw every 5 min. Mice were kept spontaneously breathing to avoid the trauma of tracheal intubation and mechanical ventilation. Respiration rates were manually counted three times for each mouse by two individuals independently. We found that respiratory rates were similar between sevoflurane (135 ± 10 per min) and propofol (122 ± 9 per min), both of which were lower than control (142 ± 10 per min). In addition, mucous membrane color was checked regularly throughout the procedure. Heart rate and blood oxygen were monitored by a pulse oximeter (Mouse STAT, Kent Scientific Corporation, USA). During the experiment, mice were placed on heating pads, and body temperature was monitored by a probe (RightTemp, Kent Scientific). No analgesia was administered as the mice showed no signs of pain. All the mouse procedures were conducted according to the protocols approved by the Stony Brook University Institutional Animal Care and Use Committee.

Treatment of Endothelial Cells with Sevoflurane or Propofol

Human umbilical vein endothelial cells (HUVECs) were purchased from ATCC (USA) and maintained in vascular cell basal medium (PCS-100-030) supplemented with endothelial cell growth kit-BBE (PCS-100-040). HUVECs at passages three to six were used for experiments.

Mouse pulmonary endothelial cells (MPECs) were isolated as described previously.¹⁴ Briefly, Dynabeads sheep anti-rat IgG (11035, Invitrogen, USA) was preincubated with purified anti-mouse CD31 (platelet endothelial cell adhesion molecule [PECAM]-1, 553370, BD Pharmingen, USA) overnight before usage. A 6-week-old *balb/c* female mouse was euthanized. After perfusion, the lung was removed, minced with scissors, and digested with 1 mg/ml collagenase/dispase solution in Dulbecco's modified Eagle Medium (Thermo Fisher Scientific, USA) for 45 min at 37°C. Then the digested lung tissue was passed through a 14-gauge needle at least 12 times, spun down to precipitate cells, and incubated with anti-mouse PECAM-1 antibody-conjugated Dynabeads for 15 min at room temperature. MPECs captured by Dynabeads were separated from other cells using a magnetic separator and cultured in a gelatin-coated cell culture flask with mouse primary endothelial cell growth medium (M1168, Cell Biologics, USA).

Endothelial cells were exposed to either air or sevoflurane in air using an airtight glass chamber with inlet and outlet connectors. The chamber atmosphere was saturated with water at 37°C. The inlet port of the chamber was connected to the anesthesia machine (Matrix VIP 3000, Sevoflurane Vaporizer, USA) and flushed with 3.3% sevoflurane mixed with 95% air/5% CO₂ at 0.5 l per min, and sevoflurane-saturated culture medium was added to the cells. Sevoflurane concentrations were monitored at the chamber exit port

using an anesthesia gas monitor (POET IQ, Criticare Systems Inc., USA). For exposure to propofol, pure propofol (2,6-diisopropylphenol, D126608, Sigma-Aldrich, USA) was diluted with dimethyl sulfoxide (DMSO) to 4 mg/ml and added to culture cells to a final concentration of 4 µg/ml.

In Vitro Endothelial Permeability Assay

Transparent polyethylene terephthalate transwell inserts with 1-µm pores were precoated with a thin layer of type I collagen, and 100,000 HUVECs or MPECs were seeded on top. The confluent endothelial monolayer was treated different concentrations of sevoflurane (0.1, 0.15, 0.2, and 0.3 mM) or propofol (1, 4, and 10 µg/ml) for 4 h. After treatments, fluorescein isothiocyanate (FITC)-dextran was added to the inserts and incubated for 1 h, and fluorescence in the receiver well was measured by a Perkin Elmer (USA) Victor X3 2030 multimode microplate plate reader with filters for excitation of 485 nm and emission of 535 nm. After the FITC-dextran permeability test, the endothelial monolayer was stained with crystal violet for brightfield imaging.

mRNA Sequencing and Analysis

HUVECs were treated with sevoflurane or propofol for 4 h *in vitro*. After treatment, total RNA was extracted from those cells and subject to mRNA sequencing (mRNA-seq) using an Illumina NovSeq 6000 Sequencer (Illumina, USA) in the Genomic Resources Core Facility at Weill Cornell Medicine (New York, New York). Genes that were differentially expressed between two conditions (sevoflurane *vs.* propofol) were identified according to the fold change (greater than 2), and statistical significance was determined by unpaired Student *t* test followed by Benjamini-Hochberg correction ($P < 0.05$). Activated pathways were enumerated by uploading the differentially expressed gene to the gene ontology (GO) package for enriched biologic processes and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using the Fisher exact test for enriched pathways ($P < 0.05$). Gene set enrichment analysis enrichment plots based on MSigDB 7.5 (Broad Institute, USA) were generated using gene set enrichment analysis software with the default parameters.¹⁵ The mRNA-seq data are accessible in the gene expression omnibus (GEO) database.

Quantitative Real-time Polymerase Chain Reaction and Hypoxia-inducible Factor 1α Knockdown by siRNA

Total RNA was extracted from endothelial cells using the RNeasy mini-kit (74104, Qiagen, Germany) and cDNA first-strand synthesis with SuperScript IV Reverse Transcriptase (18090050, Thermo Fisher Scientific). Real-time polymerase chain reaction was performed using PowerUp SYBR Green Master Mix (A25742, Thermo Fisher Scientific) and ABI 7300 PCR System (Applied Biosystems, USA) with primer pairs for human or murine hypoxia-inducible factor (HIF) isoforms and human

glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or mouse β-actin (table 1). The delta-delta Ct methods using GAPDH or β-actin as a reference were utilized to compute the relative expression of HIF isoforms. The procedures were performed three times using independent samples.

Murine HIF-1α small interfering RNA (siRNA) (M-040638-00, Dharmacon siGenome SMARTpool) and corresponding control siRNA (D-001206-14-20, Dharmacon siGenome Control pool) were purchased from Horizon Discovery (United Kingdom) and applied to MPECs according to the manufacturer's instructions.

Western Blot Analysis

Endothelial cell lysate or lung lysate obtained from mice was subjected to Western blot analysis. Cell lysate or lung lysate (30 µg) was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 10% gel and transferred to a methanol-activated polyvinylidene fluoride membrane. For detection of HIF-1α, HIF-2α, and GAPDH, rabbit monoclonal anti-HIF-1α (1:1,000, NB100-479, Novus Biologicals, USA), rabbit monoclonal anti-HIF-2α (NB100-132, Novus Biologicals), and rabbit monoclonal anti-GAPDH (1:1,000, 2118S, Cell Signaling, USA) were used, followed by a goat anti-rabbit IgG-horseradish peroxidase secondary antibody (1:5,000, sc-2004, Santa Cruz Biotechnology, USA). Antibody binding was visualized with enhanced chemiluminescence.

Immunofluorescent Staining

HUVEC or MEPC cells were grown to confluence in 35-mm glass-bottom petri dishes, exposed to 0.2 mM sevoflurane or 4 µg/ml propofol for 4 h, and then fixed with 2% paraformaldehyde for 20 min. After blocking/permeabilization in blocking buffer (2% normal goat serum, 0.2% Triton X-100, Sigma-Aldrich) for 1 h, fixed cells were labeled with mouse anti-human vascular endothelial (VE)-cadherin antibody (1:200, 555661, BD Pharmingen) or rabbit monoclonal anti-HIF-1α (1:1,000, NB100-479, Novus Biologicals) overnight at 4°C. The next day, cells were incubated with Alexa Fluor (Thermo Fisher Scientific) 488 goat anti-mouse IgG for 1 h at room temperature. Filamentous actin (F-actin) staining was performed using Texas Red phalloidin (T7471, Thermo Fisher Scientific), and the nucleus was stained with 4',6-diamidino-2-phenylindole (DAPI) in ProLong Gold Antifade Mountant (P36931, Thermo Fisher Scientific). Fluorescent microscopic sections were acquired and processed by fluorescence microscope with a Zeiss (USA) LSM 980 Airyscan 2 NLO Two-Photon Confocal Microscope. Five randomly selected areas were acquired at 20× or 60× magnification from each section and processed with Zen 3.6 (Zeiss, Germany) (blue edition). All images shown are representative of three independent experiments.

To quantify endothelial integrity, the percentage of the area not covered by HUVECs was calculated from the microscopic fluorescent section obtained by VE-cadherin staining. To do

Table 1. Primers for Quantitative Real-time Polymerase Chain Reaction

Gene Name	Sense (5'–3')	Antisense (5'–3')
Human HIF-1 α	CATAAAGTCTGCAACATGGAAGGT	ATTGATGGGTGAGGAATGGGT
Human HIF-2 α	GCGCTAGACTCCGAGAACAT	TGGCCACTTACTACCTGACCCCTT
Human HIF-3 α	GTCGGAGAGTATCGTCTGTGTC	TCTGCCGAGAGTGTTGCTCCGTT
Human GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTTC
Mouse HIF-1 α	CTATGGAGGCCAGAAGAGGGTAT	CCCACATCAGGTGGCTCATAA
Mouse HIF-2 α	CTGAGGAAGGAGAAATCCCGT	TGTGTCCGAAGGAAGCTGATG
Mouse HIF-3 α	GGAGGCTATCTGTGGACTCAGA	TGTTCCAGAGACAGCACCCTC
Mouse β -actin	TGGACAGTGACATCAAAAG	TCGTGCGTGACATCAAAAG

HIF, hypoxia-inducible factor.

that, a single grayscale image for VE-cadherin and F-actin channel was extracted using ImageJ (National Institutes of Health, USA), and lower and upper threshold values were manually set for each image. Automatic area measurements were used to calculate the percentage of the area not covered by HUVECs. The average of area percentage from combined VE-cadherin and F-actin channels was used for each microscopic section.

Animal Model of Exposure to General Anesthetics and *In Vivo* Permeability Assay

Balb/c mice of both sexes were used in the *in vivo* permeability assay. General anesthesia was administered with 3.3% sevoflurane or an intraperitoneal injection of propofol, with an initial dose of 150 mg/kg and a bolus of 50 mg $\cdot$ kg⁻¹ $\cdot$ h⁻¹ for 3 h. During general anesthesia, temperature was controlled, and the mice were monitored with a pulse oximeter. Oxygen was administered when oxygen saturation dropped to less than 95%.

For *in vivo* permeability assay, 100 μ l AngioSense 750 (NEV10011EX) was injected into a tail vein during general anesthesia exposure. One day after exposure, the mouse lung was harvested for imaging with a bioluminescent *in vivo* Imaging System (IVIS) machine (IVIS Lumina III, PerkinElmer, USA) and prepared for paraffin sectioning. A sampling of sections was taken across the lung and stained with hematoxylin and eosin. In the second cohort of mice, 10 kDa rhodamine-dextran (D1824, Thermo Fisher Scientific) was injected into the tail vein at 100 mg/kg before general anesthesia. After 4 h of general anesthesia as described in the previous paragraph, each mouse was perfused with saline followed by 5 ml 4% formaldehyde. The lung was cryosectioned and stained with *Lycopersicon esculentum* lectin. Vascular leakage was examined by a Zeiss LSM 980 Airyscan 2 NLO Two-Photon Confocal Microscope. Five randomly selected areas were acquired at 20 $\times$ magnification from each section and processed with Zen 3.6 (blue edition). All images shown are representative of three independent experiments.

VEGF Enzyme-linked Immunosorbent Assay

Mouse blood was collected for serum, and lungs were harvested and digested with lysis buffer (78510, T-PER Tissue

Protein Extraction Reagent, Thermo Fisher Scientific) containing protease inhibitors (04693159001, cOmplete Protease Inhibitor Cocktail, Roche, USA). The protein concentration of tissue lysate was determined by Bicinchoninic Acid Assay (23225, Pierce BCA Protein Assay, Thermo Fisher Scientific). Serum and tissue lysate were subjected to enzyme-linked immunosorbent assay (ELISA) for VEGF according to the manufacturer's instructions (DY493-05, Mouse VEGF DuoSet, R&D Systems, USA). The VEGF concentrations in serum and lung were calculated according to the volume of serum and the protein concentration of lung tissue lysate, respectively.

Statistical Analysis

GraphPad Prism (version 9) was used to calculate statistics. The Shapiro–Wilk test was used to assess normality of data. Normally distributed data were analyzed by Student *t* test or one-way ANOVA for repeated measures followed by the Dunnett *post hoc* test. Nonnormally distributed data were analyzed using the Mann–Whitney U test or Kruskal–Wallis test with multiple comparisons (Dunn test). For all tests, $P < 0.05$ was considered statistically significant. Means and SDs are shown in the figures.

For animal study, the primary outcome is the *ex vivo* fluorescence intensity of AngioSense 750 in lung. Estimate measure of control group was fluorescence signal intensity of 3.11E+09 with SD of 5.48E+08. No significant change was expected for the propofol group and an increase of more than 30% was estimated for the sevoflurane group based on our previous study, which showed the difference in the burden of breast cancer lung metastasis between sevoflurane and propofol was greater than 30%.⁸ Using $\alpha = 0.05$ and 80% power, we estimated a total sample size of 16 for three groups (JMP by SAS, USA version 10.0.0).

Effect size and CI for animal studies is calculated using Python 3.11.11, Pandas 2.2.2, Numpy 1.26.4, and Scipy 1.13.1. The statistical tests and biologic replicate numbers for each experiment are presented in each figure legend. All data collected from animal and cell experiments were included in the analyses.

Results

Sevoflurane, but Not Propofol, Disrupted the Integrity of HUVEC Monolayers

One of the essential functions of endothelial cells is to maintain vascular homeostasis by forming a selectively permeable layer. To assess how general anesthetics affect the endothelial integrity *in vitro*, we employed a widely used functional assessment of tight junction permeability, the paracellular tracer permeability assay, with the HUVEC monolayers. The concentrations of general anesthetics were chosen based on the clinical concentrations reported in literature. Sevoflurane at 3.3% (0.2 mM) is equivalent to 1.5 minimum alveolar concentration (MAC) for surgical anesthesia.¹⁶ For propofol, the plasma concentration to maintain surgical anesthesia is 4 to 6 µg/ml.¹⁷ Sevoflurane induced a significant increase of HUVEC permeability to 3 kD dextran at 0.2 and 0.3 mM but no effect at 0.1 and 0.15 mM (fig. 1A; the measurements of fluorescence intensity in all samples were normalized to control and presented as fold change; 0.1 mM, 1.13 ± 0.16 ; 0.15 mM, 1.43 ± 0.18 ; 0.2 mM, 1.86 ± 0.34 ; 0.3 mM, 2.48 ± 0.66). Propofol showed no significant effect on HUVEC permeability across different concentrations (fig. 2A; DMSO, 1.01 ± 0.08 ; 1 µg/ml, 1.07 ± 0.06 ; 4 µg/ml, 0.99 ± 0.14 ; 10 µg/ml, 1.10 ± 0.12). None of the propofol concentrations tested in figure 1A affected HUVEC viability as shown in Supplemental Digital Content figure 1 (<https://links.lww.com/ALN/D897>). In the second set of experiments, the HUVEC permeability in response to sevoflurane (0.2 mM) or propofol (4 µg/ml) at different timepoints was tested. Treatment with sevoflurane for more than 3 h induced a significant increase of transendothelial permeability (fig. 1B; 1 h, 1.19 ± 0.07 ; 2 h, 1.21 ± 0.15 ; 3 h, 1.47 ± 0.18 ; 4 h, 1.74 ± 0.35 ; 6 h, 2.10 ± 0.24 ; 24 h, 2.67 ± 0.53). Treatment with propofol had no effect on endothelial integrity (fig. 1B; 1 h, 0.96 ± 0.09 ; 2 h, 1.04 ± 0.06 ; 3 h, 1.10 ± 0.06 ; 4 h, 1.04 ± 0.16 ; 6 h, 1.08 ± 0.08 ; 24 h, 1.12 ± 0.1). To validate the results obtained in the transendothelial permeability assay, we measured the transendothelial electrical resistance using a volt/ohm meter as illustrated in figure 1C. Sevoflurane but not propofol significantly reduced the electrical resistivity of HUVECs ($n = 5$; control, 33.48 ± 5.26 ; sevoflurane, 9.27 ± 2.19 ; DMSO, 34.51 ± 4.26 ; propofol, $39.62 \pm 3.97 \Omega \cdot \text{cm}^2$; control *vs.* sevoflurane, $P = 0.02$). Endothelial permeability is tightly regulated, in part, by the endothelial adherent junctions, which are largely composed of VE-cadherin.¹⁸ We performed immunofluorescence staining of VE-cadherin of the HUVEC monolayer to examine its integrity upon treatment with sevoflurane or propofol. Both anesthetics disrupted intercellular junctions after 4-h incubations, but sevoflurane had a significantly greater effect than propofol (fig. 1D; $n = 4$; control, 0.43 ± 0.57 ; sevoflurane, 17.86 ± 2.08 ; DMSO, 0.78 ± 0.61 ; propofol, 4.18 ± 1.05 ; control *vs.* sevoflurane, $P = 0.02$). Moreover, costaining of cellular

F-actin showed that sevoflurane induced a 1.5-fold increase of HUVEC F-actin, indicating significant cytoskeletal rearrangements in HUVECs (fig. 1D; $n = 5$; control, $1,111.88 \pm 96.41$; sevoflurane, $1,524.49 \pm 209.02$; DMSO, $1,159.45 \pm 80.92$; propofol, $1,226.11 \pm 119.76$; control *vs.* sevoflurane, $P = 0.03$). Thus, decreased resistivity and increased migration of FITC-dextran through the HUVEC layer induced by sevoflurane correlated well with the disassembly of VE-cadherin by sevoflurane.

Sevoflurane Increased Vascular Permeability via HIF-1 α /VEGF

To determine the molecular mechanism underlying anesthetic-mediated changes in endothelial cells, we employed mRNA-seq analysis to assay the change in gene expression of HUVECs treated with sevoflurane or propofol. At a fold-change cutoff of greater than 2 or less than -2, there were 368 genes either upregulated or downregulated in HUVECs treated with sevoflurane, while only 36 genes changed the expression in HUVECs treated with propofol (fig. 2A; full list of genes differentially changed is shown in Supplemental Digital Content table 2, <https://links.lww.com/ALN/D899>). The gene expression patterns in HUVECs treated with sevoflurane or propofol were significantly different (fig. 2B; 3% of genes upregulated in common, 7% of genes downregulated in common). Gene set enrichment analysis on these datasets was performed to screen for pathways and processes. The analysis indicates that sevoflurane treatment resulted in changes of multiple cellular processes, including tumor necrosis factor alpha (TNF α) signaling *via* nuclear factor kappa b (NF κ B), hypoxia, and hedgehog signaling, all of which are actively involved in vascular remodeling (Supplemental Digital Content fig. 2, <https://links.lww.com/ALN/D897>; enrichment with false discovery rate (FDR) less than 0.25, $P < 0.01$). Among the genes identified in our mRNA-seq datasets, three HIF- α isoforms, HIF-1 α , -2 α , and -3 α , were upregulated (Supplemental Digital Content fig. 2, <https://links.lww.com/ALN/D897>). To validate the mRNA-seq results, we first confirmed the changes of HIF- α expressions induced by general anesthetics with real-time polymerase chain reactions in HUVECs. The HIF-1 α mRNA level was markedly increased as much as 1.7-fold after treatment with sevoflurane but not propofol (fig. 2C; $n = 4$; DMSO, 1.02 ± 0.15 ; propofol, 0.61 ± 0.19 ; sevoflurane, 1.77 ± 0.39 ; control *vs.* sevoflurane, $P = 0.03$). The HIF-2 α mRNA level was also increased after treatment with sevoflurane but not statistically significantly (DMSO, 1.09 ± 0.26 ; propofol, 0.85 ± 0.32 ; sevoflurane, 1.40 ± 0.27). The HIF-3 α mRNA level did not change in HUVECs upon anesthetic treatments (DMSO, 1.02 ± 0.12 ; propofol, 1.03 ± 0.11 ; sevoflurane, 1.36 ± 0.12). We observed an increase of both HIF-1 α and -2 α proteins in HUVECs 1 day after treatments with sevoflurane but not propofol using Western blot (fig. 2D; HIF-1 α control, 0.33 ± 0.04 ; sevoflurane,

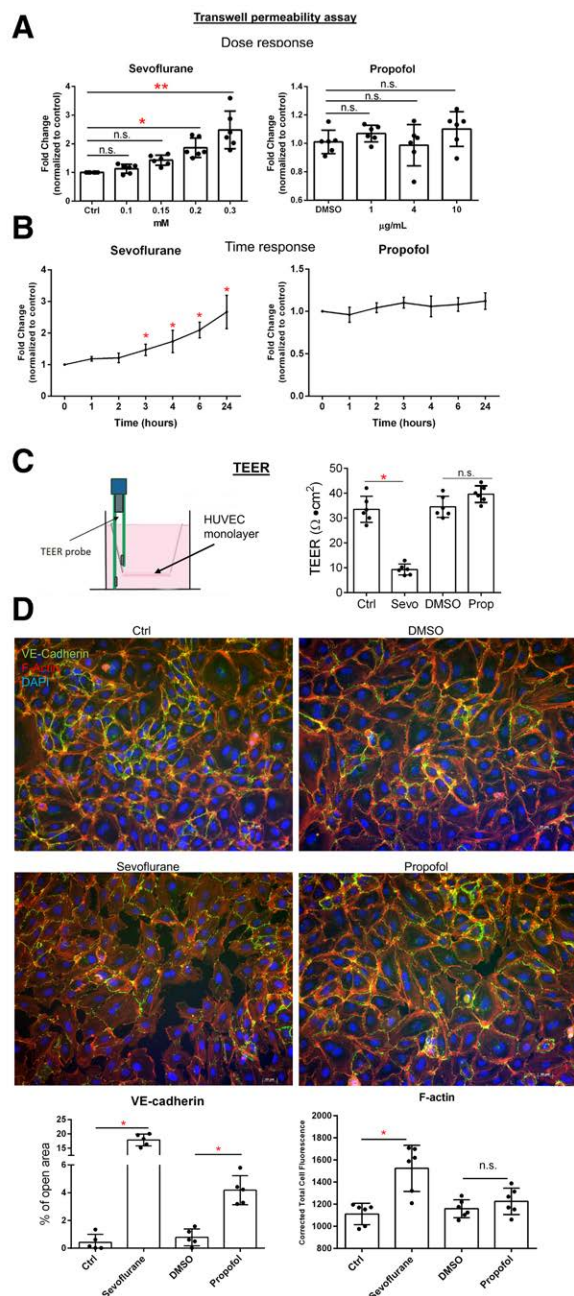


Fig. 1. Sevoflurane but not propofol disrupts the integrity of HUVECs *in vitro*. (A) Dose responses of trans-HUVEC permeability to sevoflurane or propofol. Monolayers of HUVECs formed on transparent polyethylene terephthalate (PET) membranes were exposed to sevoflurane or propofol at indicated doses or time-points. Leakage of FITC-dextran through HUVEC monolayer was quantified by fluorescence after general anesthetic exposure. Sevoflurane but not propofol induced significantly increase of the FITC-dextran leakage through HUVECs at concentrations of 0.2 and 0.3 mM for 4-h exposure ($n = 6$; for sevoflurane: control vs. 0.2 mM, $P = 0.04$; control vs. 0.3 mM, $P = 0.001$; Kruskal–Wallis test plus Dunn *post hoc* test). (B) Time responses of trans-HUVEC permeability to sevoflurane or propofol. (Continued)

0.78 $\pm$ 0.14; DMSO, 0.31 $\pm$ 0.05; propofol, 0.30 $\pm$ 0.06; $n = 4$; control vs. sevoflurane, $P = 0.001$; HIF-2 α control, 0.31 $\pm$ 0.06; sevoflurane, 0.85 $\pm$ 0.07; DMSO, 0.34 $\pm$ 0.08; propofol, 0.45 $\pm$ 0.13). Moreover, after sevoflurane exposure, there was a distinct translocation of HIF-1 α from the cytoplasm into the nucleus compared to control and propofol treatment (fig. 2E). The translocation of HIF-1 α triggers the expression of several downstream genes including VEGF.¹⁹ VEGF is the key regulator of vascular permeability. The effect of VEGF on vascular permeability has been shown to be biphasic; it produces a rapid increase followed by a longer and more sustained increase for a few days.²⁰ *In vitro* cultured HUVECs were exposed to sevoflurane or propofol for 3 h, and the levels of VEGF in culture supernatant and cell lysate were measured using ELISA at 24 h after treatment. Both the release of VEGF from HUVECs into the cell culture medium and the intracellular level of VEGF were significantly higher in the sevoflurane group compared to the control and propofol groups (fig. 2F; supernatant: control, 215.00 $\pm$ 44.41; sevoflurane, 605.16 $\pm$ 17.20; DMSO, 229.58 $\pm$ 24.06; propofol, 247.66 $\pm$ 71.94; $n = 4$; control vs. sevoflurane, $P = 0.028$; cell lysate: control, 373.19 $\pm$ 136.74; sevoflurane, 765.07 $\pm$ 131.38; DMSO, 363.87 $\pm$ 22.71; propofol, 376.34 $\pm$ 50.16; $n = 4$; control vs. sevoflurane, $P = 0.007$). Collectively, our data show that sevoflurane modulates the permeability of HUVECs and activates HIF-1 α and downstream VEGF production in HUVECs.

Sevoflurane, but Not Propofol, Increased the Permeability of MPECs *In Vitro*

We next determined the effects of general anesthetics on the function of pulmonary endothelial cells. Based on the

Fig. 1. (Continued) Sevoflurane but not propofol induced significantly increase of FITC-dextran leakage at 0.2 mM for 3-, 4-, 6-, and 24-h exposure ($n = 6$; for sevoflurane: control vs. 3-h, $P = 0.046$; control vs. 4-h, $P = 0.03$; control vs. 6-h, $P = 0.005$; control vs. 24-h, $P < 0.0001$; Kruskal–Wallis test plus Dunn *post hoc* test). (C) Transendothelial electric resistance (TEER) of HUVECs. As an indicator of endothelium integrity, TEER was measured for HUVEC exposure to sevoflurane (0.2 mM) or propofol (4 μ g/ml) for 4 h. Sevoflurane but not propofol significantly reduced TEER of HUVEC in comparison to control (DMSO as the control to propofol; $n = 6$; control vs. sevoflurane, $P = 0.02$; one-way ANOVA plus Dunnett *post hoc* test). (D) The effect of general anesthetics on VE-cadherin. The monolayers of HUVECs were incubated with sevoflurane (0.2 mM) or propofol (4 μ g/mL) for 4 h. After treatment, HUVECs were costained with VE-cadherin antibody, Texas Red Phalloidin for F-actin, and DAPI, and the gaps between cells were quantified. Sevoflurane induced significantly more gaps in HUVECs than propofol ($n = 5$; for VE-cadherin, control vs. sevoflurane, $P = 0.02$; DMSO vs. propofol, $P = 0.04$; for F-actin: control vs. sevoflurane, $P = 0.03$; one-way ANOVA plus Dunnett *post hoc* test). Values are mean $\pm$ SD. F-actin, filamentous actin; HUVEC, human umbilical vein endothelial cell; VE, vascular endothelial.

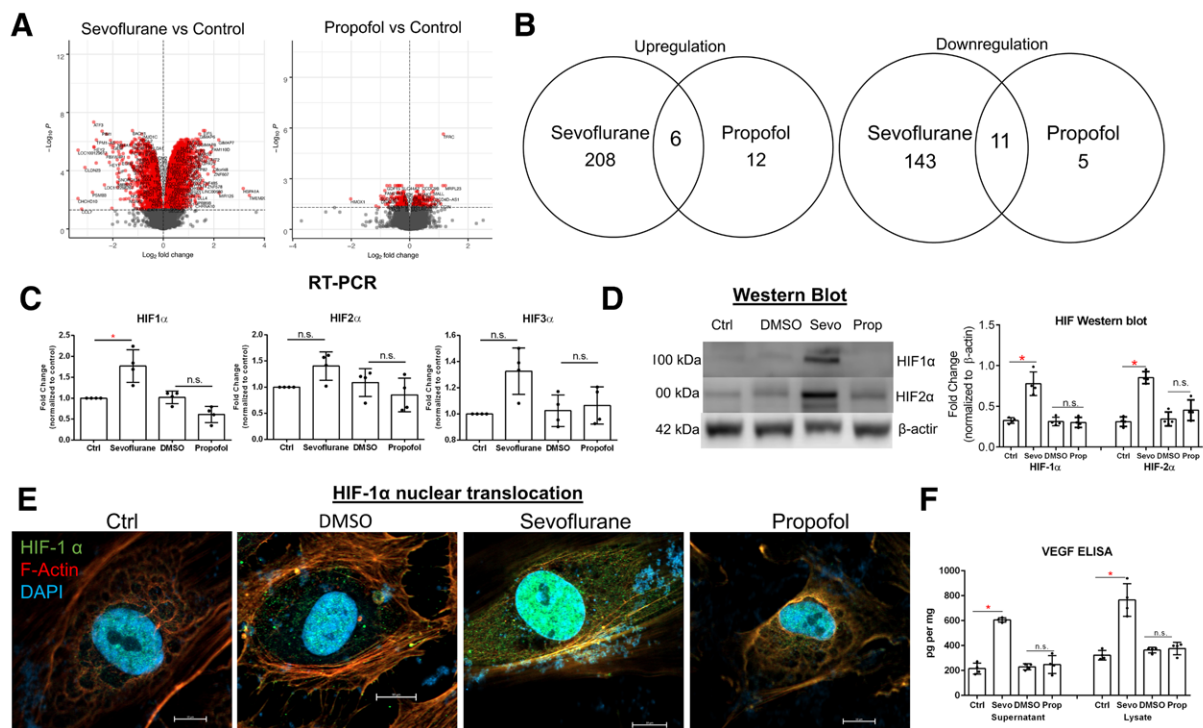


Fig. 2. Sevoflurane activates HIF-1 α and induces VEGF expression in HUVECs. (A) mRNA-sequencing analysis of HUVECs treated with sevoflurane or propofol. Volcano plots show differential expression of genes in HUVECs treated with sevoflurane (0.2 mM) or propofol (4 μ g/mL) for 4 h. (B) Venn diagrams show upregulated and downregulated genes identified by comparing expressions after sevoflurane or propofol treatment. (C) Validation of HIF- α mRNA levels in HUVECs treated with general anesthetics. Sevoflurane led to significantly increase of HIF-1 α mRNA level compared to propofol ($n = 4$; control vs. sevoflurane, $P = 0.03$; one-way ANOVA plus Dunnett *post hoc* test). (D) Western blot analysis of HIF- α isoforms in HUVECs treated with general anesthetics. Sevoflurane increased HIF-1 α and HIF-2 α protein level compared to propofol or control ($n = 4$; HIF-1 α control vs. sevoflurane, $P = 0.001$; HIF-2 α control vs. sevoflurane, $P = 0.001$; unpaired t test). (E) Subcellular localization of HIF-1 α in HUVECs treated with general anesthetics. After treatment, HUVECs were fixed and costained with HIF-1 α antibody, Texas Red Phalloidin for F-actin, and DAPI for the location of nucleus. Sevoflurane promoted the translocation of HIF-1 α from cytoplasm to nucleus. (F) Sevoflurane significantly increased VEGF levels in HUVECs compared to propofol or control ($n = 4$; supernatant control vs. sevoflurane, $P = 0.028$; Mann-Whitney test; cell lysate control vs. sevoflurane, $P = 0.007$; unpaired t test). Values are mean $\pm$ SD. F-actin, filamentous actin; HIF, hypoxia-inducible factor; HUVEC, human umbilical vein endothelial cell; VEGF, vascular endothelial growth factor.

results obtained from HUVECs, we first analyzed the effect of sevoflurane and propofol on MPEC viability. Neither anesthetic at clinically relevant concentrations affected MPEC viability (Supplemental Digital Content fig. 3, <https://links.lww.com/ALN/D897>). Then we analyzed the expressions of HIF- α isoforms in isolated MPECs. A 4-h treatment of sevoflurane but not propofol significantly increased the level of HIF-1 α mRNA (fig. 3A; HIF-1 α : DMSO, 1.12 ± 0.10 ; propofol, 0.50 ± 0.17 ; sevoflurane, 1.66 ± 0.44 ; $n = 4$; control vs. sevoflurane, $P = 0.03$). Expressions of HIF-2 α and HIF-3 α were not significantly changed by sevoflurane (HIF-2 α : DMSO, 1.08 ± 0.16 ; propofol, 0.68 ± 0.21 ; sevoflurane, 1.30 ± 0.42 ; HIF-3 α : DMSO, 1.02 ± 0.10 ; propofol, 0.66 ± 0.22 ; sevoflurane, 0.87 ± 0.17). The increase of HIF-1 α expression by sevoflurane was confirmed by Western blot (fig. 3B; HIF-1 α : control, 0.32 ± 0.03 ; sevoflurane, 0.57 ± 0.15 ; DMSO, 0.31 ± 0.05 ; propofol, 0.26 ± 0.02 ; $n = 4$; control vs. sevoflurane,

$P = 0.015$). Immunofluorescence analysis showed that sevoflurane not only augmented total HIF-1 α staining but also increased the intensity of HIF-1 α -stained nuclei, which implied the activation and translocation of HIF-1 α (fig. 3C). To test whether HIF-1 α mediated the sevoflurane-induced change in vascular permeability, we knocked down HIF-1 α in MPECs using siRNA, which was confirmed by Western blot (fig. 3D; HIF-1 α scramble RNA: control, 0.30 ± 0.03 ; sevoflurane, 0.57 ± 0.15 ; DMSO, 0.33 ± 0.04 ; propofol, 0.26 ± 0.02 ; $n = 4$; control vs. sevoflurane, $P = 0.01$). HIF-1 α knockdown reduced the secretion of VEGF induced by sevoflurane in MPECs (fig. 3E; HIF-1 α scramble RNA supernatant: control, 184.73 ± 29.68 ; sevoflurane, 597.04 ± 72.55 ; DMSO, 188.22 ± 21.05 ; propofol, 306.00 ± 82.22 ; $n = 5$; control vs. sevoflurane, $P = 0.002$; HIF-1 α scramble RNA cell lysate: control, 385.78 ± 55.24 ; sevoflurane, 678.83 ± 57.88 ; DMSO, 373.63 ± 52.87 ; propofol, 368.72 ± 108.90 ; $n = 5$; control vs. sevoflurane, $P =$

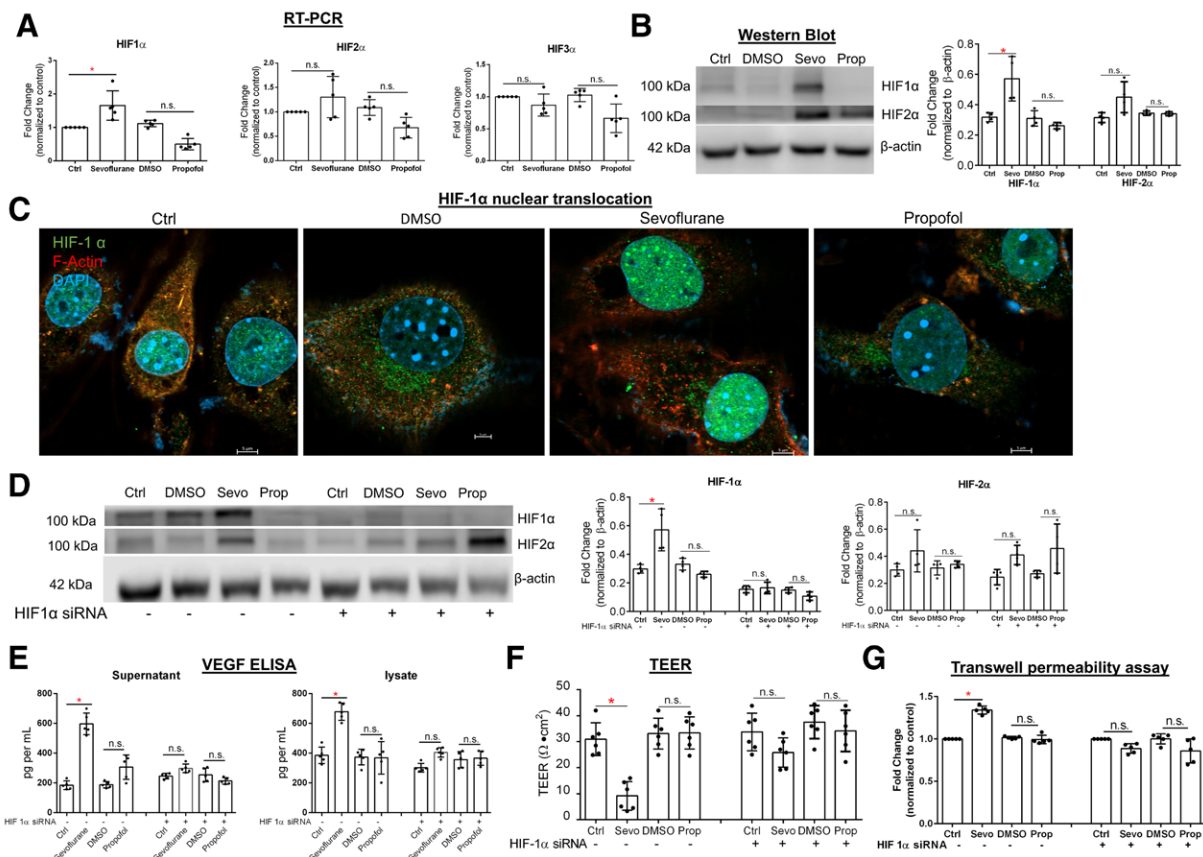


Fig. 3. Sevoflurane mediates activation of HIF/VEGF signaling and disrupts the integrity of MPECs. MPECs were isolated from 6-week-old C57BL/6 mice and subjected to treatment with sevoflurane (0.2 mM) or propofol (4 $\mu\text{g}/\text{ml}$) for 4 h. (A) RT-PCR and (B) Western blot analysis of HIF- α isoforms in MPECs after sevoflurane and propofol exposures. Sevoflurane remarkably increased HIF-1 α level compared to control or propofol in MPECs (RT-PCR: $n = 5$; control vs. sevoflurane, $P = 0.03$; one-way ANOVA plus Tukey *post hoc* test; Western blot: $n = 4$; control vs. sevoflurane, $P = 0.015$; unpaired *t* test). (C) Immunofluorescence of HIF-1 α subcellular localization in MPECs. Sevoflurane induced HIF-1 α translocation from cell cytosol to nucleus in MPECs. (D) The knockdown of HIF-1 α by siRNA prevented the increased expression of HIF-1 α induced by sevoflurane as evidenced by Western blot ($n = 4$; control vs. sevoflurane, $P = 0.01$; unpaired *t* test). (E) Sevoflurane increased VEGF levels in the supernatant and lysate of MPECs, which were abolished by HIF- α siRNA ($n = 5$; supernatant: control vs. sevoflurane, $P = 0.002$; cell lysate: control vs. sevoflurane, $P = 0.01$; one-way ANOVA plus Dunnett *post hoc* test). (F) *In vitro* transwell model with MPECs. On MPEC monolayers formed on transparent polyethylene terephthalate (PET) membranes, sevoflurane resulted in a significant decrease in TEER compared to propofol or control fluorescence ($n = 6$; control vs. sevoflurane, $P = 0.02$; one-way ANOVA plus Dunnett *post hoc* test) and (G) increased FITC-dextran migration as quantified by fluorescence ($n = 5$; control vs. sevoflurane, $P = 0.02$; one-way ANOVA plus Dunnett *post hoc* test). The effects of sevoflurane were abolished by HIF- α siRNA. Values are mean $\pm$ SD. HIF, hypoxia-inducible factor; MPEC, mouse pulmonary endothelial cell; RT-PCR, real-time polymerase chain reaction; TEER, transendothelial electric resistance; VEGF, vascular endothelial growth factor.

0.01). Sevoflurane did not change the transendothelial electrical resistance of HIF-1 α knockdown cells (fig. 3F; scramble RNA: control, 30.95 ± 6.31 ; sevoflurane, 9.17 ± 5.54 ; DMSO, 33.12 ± 5.93 ; propofol, $33.38 \pm 6.15 \Omega \cdot \text{cm}^2$; $n = 6$; control vs. sevoflurane, $P = 0.02$) and transendothelial migration of FITC-dextran in HIF-1 α -silenced cells upon treatment (fig. 3G; scramble RNA: sevoflurane, 1.34 ± 0.05 ; DMSO, 1.02 ± 0.01 ; propofol, 0.99 ± 0.05 fold-change; $n = 5$; control vs. sevoflurane, $P = 0.02$). Thus, sevoflurane-induced MPEC permeability increase was HIF-1 α -dependent.

Sevoflurane, but Not Propofol, Induced Excessive Pulmonary Vascular Permeability in Mice

We further tested the effects of general anesthetics on permeability *in vivo*. We first assessed the permeability of pulmonary vasculature in mice with a fluorescent vascular imaging agent, AngioSense 750 (fig. 4A). AngioSense 750 was injected into the tail vein of mice (three male and three female mice in each group) followed by a 4-h exposure to general anesthetics. One day after anesthetic exposure, the lung was harvested for imaging. Accumulation of

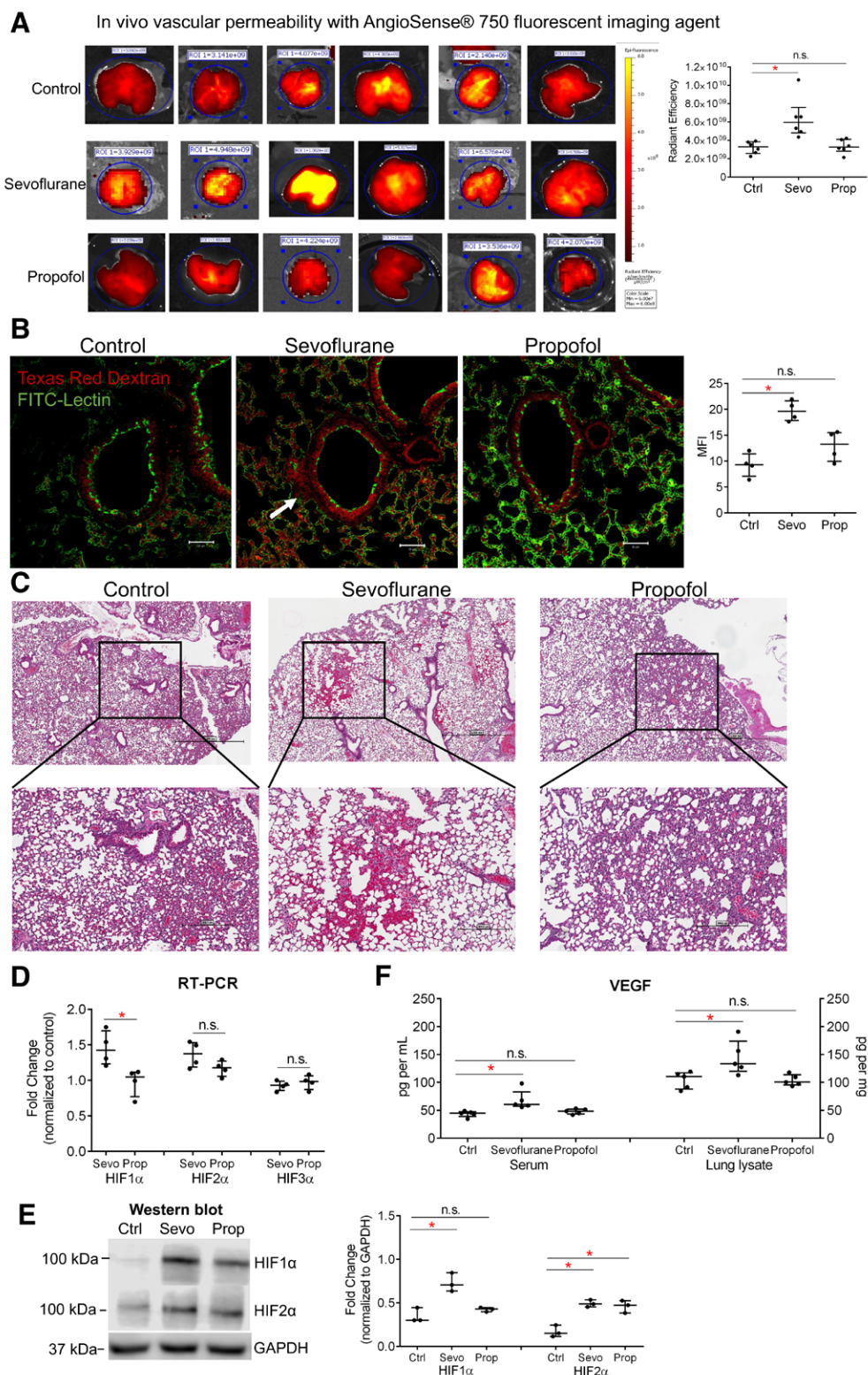


Fig. 4. Sevoflurane activated HIF-1 α /VEGF signaling and promoted pulmonary vascular permeability *in vivo*. *Balb/c* mice (6 weeks old, three male and three female) were exposed to sevoflurane (1.5 minimum alveolar concentration) or propofol (150 mg/kg followed by 50 mg/kg/h) for 4 h. (A) Sevoflurane increased vascular permeability in lungs. Mice were injected with 150 μ l AngioSense 750 fluorescent imaging agent 1 day before anesthesia exposure. After exposure, mice were euthanized, and lung was imaged with an IVIS machine to (Continued)

AngioSense 750 in the lung indicates vascular leakage. The fluorescence intensity in the lungs of the mice treated with sevoflurane was 1.8-fold higher than control, whereas propofol had no effect (control $[3.21 \pm 0.66] \times 10^9$ vs. sevoflurane $[6.40 \pm 2.25] \times 10^9$ vs. propofol $[3.34 \pm 0.76] \times 10^9$ radiant efficiency; $n = 6$ for each group; a total of 18 mice; control vs. sevoflurane, $P = 0.04$). The effect size as eta-square equals 0.63 (95% CI, 0.63 to 0.81). This represents a large effect size, suggesting a substantial proportion of variance in AngioSense 750 fluorescent signal is explained by group membership. To validate this observation, we treated another cohort of mice with rhodamine-dextran. During exposure to general anesthetics, rhodamine-dextran was injected into a tail vein. One day later, the lung was harvested and perfused with saline to eliminate circulating dextran, then cryosectioned to visualize the diffusion of rhodamine-dextran into the tissue. The pulmonary vasculatures were counterstained with *Lycopersicon esculentum* lectin. Similar to the result with AngioSense 750, we found that remarkably more rhodamine-dextran was released into lung tissue in the sevoflurane group than the control or propofol group (fig. 4B; control, 9.28 ± 2.30 vs. sevoflurane, 19.69 ± 1.97 vs. propofol, 12.92 ± 2.96 mean fluorescence intensity (MFI); $n = 4$ for each group; a total of 12 mice; control vs. sevoflurane, $P = 0.002$). In addition, upon light microscopic examination, vascular changes were extensive in sevoflurane-treated mice, with necrosis with blood and exudate in the lung parenchyma (fig. 4C; Supplemental Digital Content fig. 4, <https://links.lww.com/ALN/D897>), which indicate leakage and proinflammation of the endothelium.

We then examined the role of HIF-1 α in the change of pulmonary vascular permeability *in vivo*. Sevoflurane, but not propofol, significantly increased the expression of HIF-1 α in the lung as determined by real-time polymerase chain reaction (fig. 4D; sevoflurane, 1.51 $\pm$ 0.28-fold vs. propofol, 0.86 $\pm$ 0.21-fold; $n = 4$ for each group; a total of 12 mice; control vs. sevoflurane, $P = 0.04$) and Western blot (fig. 4E; control, 0.35 $\pm$ 0.08-fold vs. sevoflurane, 0.73 $\pm$ 0.11-fold vs. propofol, 0.43 $\pm$ 0.02-fold; $n = 3$ for each group; a total of 9 mice; control vs. sevoflurane, $P = 0.03$). No significant difference was observed in HIF-2 α expression level between sevoflurane and propofol (fig. 4, D and

E). Furthermore, ELISA analysis shows that sevoflurane markedly increased the VEGF levels in both serum and lung tissue (fig. 4F; serum: control, 43.64 ± 5.45 pg/ml vs. sevoflurane, 68.16 ± 17.38 pg/ml vs. propofol, 48.08 ± 4.71 pg/ml; $n = 5$ for each group; a total of 15 mice; control vs. sevoflurane, $P = 0.05$; lung lysate: control, 104.36 ± 15.31 pg/mg vs. sevoflurane, 144.43 ± 30.51 pg/mg vs. propofol, 103.91 ± 9.84 pg/mg; $n = 5$ for each group; a total of 15 mice; control vs. sevoflurane, $P = 0.047$). Taken together, the results showed that sevoflurane increased vascular permeability by activating the HIF-1 α /VEGF pathway with associated structural change of endothelial cells.

Discussion

In this study, we report the significant and distinct effects of two commonly used general anesthetics on vascular permeability and reveal the underlying molecular mechanism. Specifically, we have demonstrated that sevoflurane induced endothelial and pulmonary vascular leakage through activation of HIF-1 α /VEGF in multiple cell assays and a mouse model. The perturbation of endothelial functions by general anesthetics is of possible clinical relevance to patient outcomes, and understanding the mechanism potentially leads to advances in clinical practice and improved outcomes.

Effects of Sevoflurane and Propofol on Endothelial and Pulmonary Vascular Permeability

An important effect of general anesthetics on endothelial cells is altering vascular permeability. Because endothelial integrity is the key determinant of vascular permeability, we used transendothelial permeability model with HUVECs. HUVEC is the most widely used cell model to study the physiologic function of endothelium and is relevant to many translational applications such as cardiovascular and metabolic diseases.²¹ Although the structures of vasculatures in specific organs are different, our finding with HUVECs is consistent with those with MPECs and *in vivo* pulmonary vascular permeability, which suggest that the effects of anesthetics might apply to the entire endothelium.

Dysfunction of pulmonary vasculature is implicated in many diseases, such as pulmonary edema and cancer

Fig. 4. (Continued) quantify AngioSense 750 fluorescence. Sevoflurane markedly increased the leakage of AngioSense 750 into lung tissue ($n = 6$; control vs. sevoflurane, $P = 0.04$; Kruskal-Wallis test plus Dunn *post hoc* test). (B) Pulmonary vascular permeability with dextran. Mice were injected with 100 μ l rhodamine-labeled 3-kDa dextran through tail veins before anesthesia exposure. After exposure, mice were euthanized, and lung tissues were imaged for extravagated dextran (red). Sevoflurane significantly promoted the leakage of dextran ($n = 4$; control vs. sevoflurane, $P = 0.002$; one-way ANOVA plus Dunnett *post hoc* test). (C) Hematoxylin and eosin staining of lung morphology 1 day after exposure to general anesthetics. More necrosis with blood and leakage into lung tissue were observed in sevoflurane group. (D) HIF- α mRNA levels and (E) protein levels in the mouse lungs. Sevoflurane significantly increased HIF-1 α and HIF-2 α expression in lung (RT-PCR: $n = 4$; control vs. sevoflurane, $P = 0.04$; one-way ANOVA plus Dunnett *post hoc* test; Western blot: $n = 3$; control vs. sevoflurane, $P = 0.03$; one-way ANOVA plus Dunnett *post hoc* test). (F) Sevoflurane significantly increased VEGF levels in both serum and lung tissue in the treated mice compared to propofol and control ($n = 5$; serum control vs. sevoflurane, $P = 0.04$; lung lysate control vs. sevoflurane, $P = 0.03$; two-way ANOVA plus Dunnett *post hoc* tests). VEGF levels in serum and lung tissue were measured by ELISA. Values are mean $\pm$ SD. ELISA, enzyme-linked immunosorbent assay; HIF, hypoxia-inducible factor; RT-PCR, real-time polymerase chain reaction; VEGF, vascular endothelial growth factor.

metastasis. Therefore, we tested the effect of general anesthetics on pulmonary vascular permeability using *in vitro* models with MPECs and a mouse model. We showed that sevoflurane increased the permeability of pulmonary vasculature as indicated by increased fluorescent dye and dextran trapping into surrounding tissues *in vivo*. It has also been reported that isoflurane increases vascular permeability in isolated rat lung²² and exacerbated brain edema in a mouse model of traumatic head injury.²³

In contrast to sevoflurane, within clinically relevant concentrations, propofol did not significantly affect vascular permeability in either cell monolayer or mouse models. However, at clinical overdose concentrations ($10 \text{ mg} \cdot \text{mouse}^{-1} \cdot \text{h}^{-1}$), it has been reported that propofol increased vascular permeability in mice by reducing the expression of endothelial glycocalyx in multiple organs, including peritoneal vessels, lungs, and kidneys.²⁴ Thus, the effect of propofol could be dose-dependent.

Signal Transduction Pathway Mediating the Effect of Sevoflurane-induced Vascular Leakage

Our experiments suggest a molecular mechanism by which sevoflurane may increase vascular permeability. RNA sequencing of HUVECs exposed to general anesthetics identified HIF-1 α as a candidate target. We hypothesized that activation of HIF-1 α with subsequent expression of VEGF by sevoflurane is sufficient to disrupt the integrity of pulmonary endothelium. This has not been reported previously. Our data show that sevoflurane induced HIF-1 α expression and nuclear translocation in HUVECs and MPECs. Notably, as a downstream effector protein of HIF-1 α , VEGF production was also increased in HUVECs and MPECs upon sevoflurane exposure. The role of HIF-1 α in VEGF and permeability was validated by knockdown of HIF-1 α . Furthermore, *in vivo* experiments showed that sevoflurane increased HIF-1 α expression and elevated VEGF levels. The induction of VEGF expression by sevoflurane in HUVECs was also suggested by Wang *et al.*²⁵ In addition, VEGF receptor 2 (VEGFR2) expression in HUVECs was significantly upregulated by sevoflurane.²⁵ This observation complements our finding of activation of HIF-1 α /VEGF pathway by sevoflurane, since VEGFR2 is the main receptor expressed in endothelial cells mediating the effect of VEGF. Furthermore, it has been observed in clinical studies that increased levels of VEGF were associated with sevoflurane anesthesia in surgical cancer patients. Galoş *et al.* reported that for patients who underwent breast cancer surgery, sevoflurane was associated with higher levels of VEGF compared to propofol.²⁶ Sen *et al.* found that for patients receiving radical resection of lung cancer, the combination of regional anesthesia and propofol was associated with lower levels of VEGF than inhalational anesthesia with sevoflurane.²⁷

We observed no significant effect of propofol on HIF-1 α expression and translocation. Takabuchi *et al.* found that propofol selectively inhibited HIF-1 α translocation and

suppressed HIF-1 α protein synthesis under certain hypoxic conditions.²⁸ An important aspect of our experimental design is that it does not involve hypoxic or ischemic conditions; thus, the results represent the direct effects of anesthetics. Regarding HIF-2 α , both anesthetics increased the expression of HIF-2 α but were not as significant as HIF-1 α . It has been reported that the loss of HIF-2 α affects vascular integrity, but the increase of HIF-2 α did not contribute to the endothelial cell permeability.²⁹

Potential Clinical Implications

Increased vascular permeability leads to more leakage of the contents of plasma, including cells from the blood circulation into underlying tissues. It has been shown that increased vascular permeability contributes to many disorders including cancer cell metastasis, asthma, diabetic macular edema, age-related macular degeneration, renal pathology, arthritis, ulcerative colitis, other chronic inflammation, cerebral edema, and psychiatric and neurodegenerative diseases. Thus, understanding the effect of general anesthetics on endothelium functions may have many potential clinical implications.

The VEGF signaling pathway is implicated in experimental hypoxic pulmonary hypertension-associated vascular leak.³⁰ As our result showed that sevoflurane regulated the VEGF signaling pathway and results in consequent pulmonary vascular permeability, its implication in pulmonary hypertension might be significant. It is realized that increased vascular permeability is a key feature of COVID-19-associated acute respiratory distress syndrome.^{12,13} It has been reported that sevoflurane reduced the incidence of postoperative pulmonary complications in lung resection surgery with one-lung ventilation,³¹ but this was not confirmed by another clinical trial with more enrolled patients, which showed no difference between inhalational anesthesia and propofol.³² Thus, how the effects of general anesthetics on endothelium function translate to clinical situations involving surgical trauma and underlying diseases remains to be answered. The interactions of anesthetics, surgery, and the patient's comorbidity need to be considered together for the best possible outcomes.

Limitations

This study is performed on multiple *in vitro* models with HUVECs, MPECs, and an *in vivo* model with *balb/c* mice. The results *in vitro* and the mouse model may not fully translate to humans as the environment of effectors is different. Propofol was administered to mice through intraperitoneal injection rather than intravenous infusion due to the technical difficulty of mouse size. Although we confirmed the depth of anesthesia by intraperitoneal injection was comparable to inhaled sevoflurane, the pharmacokinetic difference between intraperitoneal injection and intravenous infusion should be noted. While elevated Paco_2 can influence vascular tone and reactivity, its direct or indirect effect on vascular permeability is not clear. Because the respiratory rates between two groups

are similar and oxygen saturation is greater than 95% during the entire course of the experiment, we consider the contribution of PaCO_2 changes on vascular permeability to be minimal. One should be cautious to extrapolate the result with HUVACs and MPECs to other organs since the vasculatures in the end organs are different.

Conclusions

This study shows that sevoflurane but not propofol induces endothelial and pulmonary vascular leakage through activation of HIF-1 α /VEGF. The clinical necessity to administer general anesthetics to surgical patients urges more research to develop strategies to minimize any potential risks and adverse effects.

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Competing Interests

The authors declare no competing interests.

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Supplemental Digital Content

Supplemental Digital Content Figures, <https://links.lww.com/ALN/D897>

Supplemental Digital Content Figure 1. Cell viability of HUVEC exposure to sevoflurane or propofol.

Supplemental Digital Content Figure 2. Gene set enrichment analysis of mRNA-seq data obtained from HUVECs treated with sevoflurane or propofol.

Supplemental Digital Content Figure 3. Cell viability of MPEC exposure to sevoflurane or propofol.

Supplemental Digital Content Figure 4. Hematoxylin and eosin staining of lung morphology 1 day after exposure to general anesthetics.

Supplemental Digital Content Table 1. ARRIVE checklist for animal study, <https://links.lww.com/ALN/D898>

Supplemental Digital Content Table 2. List of genes differentially changed upon sevoflurane or propofol treatment, <https://links.lww.com/ALN/D899>

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