Life Sciences 222 (2019) 22–28

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Ropivacaine inhibits pressure-induced lung endothelial hyperpermeability in models of acute hypertension

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ARTICLE INFO

Keywords:
Local anesthetics
Mechanotransduction
Vascular barrier
Pulmonary edema and filtration coefficient

ABSTRACT

Aims: Increases in hydrostatic pressure results in endothelial hyperpermeability *via* eNOS-dependent pathways. Ropivacaine is known to inhibit eNOS activation and to attenuate lung injury. Herein, we sought to determine if ropivacaine regulates pressure-induced lung endothelial hyperpermeability.

Main methods: The effects of ropivacaine on lung permeability were assessed in two models of acute hypertension (AH): the isolated perfused lung preparation where acute increases in left atrial pressure model the hemodynamic changes of severe hypertension, and an animal model of AH induced by norepinephrine. In the IPL model, whole lung filtration coefficient (K_f) was used as the index of lung permeability; pulmonary artery pressure (P_{pa}), pulmonary capillary pressures (P_{pc}), and zonal characteristics (ZC) were measured to assess the effects of ropivacaine on hemodynamics and their relationship to K_{f2}/K_{f1}. *In vivo*, ropivacaine effects were investigated on indices of pulmonary edema (changes in P_aO_2, lung wet-to-dry ratio), changes in plasma volume and nitric oxide (NO) production.

Key findings: Ropivacaine provided robust protection from pressure-dependent barrier failure; it inhibited pressure-induced increases in K_f without affecting P_{pa}, P_{pc} or ZC. *In vivo*, ropivacaine prevented pressure-induced lung edema and associated hyperpermeability as evidence by maintaining P_aO_2, lung wet-to-dry ratio and plasma volume in levels similar to sham rats. Ropivacaine inhibited pressure-induced NO production as evidenced by decreased lung nitro-tyrosine content when compared to hypertensive lungs.

Significance: Collectively these data show that ropivacaine inhibits pressure-induced lung endothelial hyperpermeability and suggest that ropivacaine may be a clinically useful agent to prevent endothelial hyperpermeability when pulmonary pressure is acutely increased.

1. Introduction

Endothelial mechanotransduction allows the vascular wall to respond and adapt to changing mechanical forces associated with sympathetic activity, metabolic demands and other physiological stresses. Flow-dependent activation of endothelial nitric oxide synthase (eNOS) is the prototypical example of endothelial mechanotransduction [1,2], where nitric oxide (NO) promotes vasodilation as an adaptive response to increased wall shear stress. Similarly, acute increases in hydrostatic pressure also activate eNOS in endothelial cells [3]. Increases in endothelial NO induce nitrosylation of adherence junction proteins and disassembly of the junction causing endothelial hypermeability [4,5].

Controlling lung endothelial permeability has important therapeutic potential. For example, preventing pulmonary edema associated with hypertensive emergencies, acute heart failure and post-pneumonectomy syndrome could significantly reduce morbidity. Local anesthetics (LA) have well known anti-inflammatory properties including prevention of endothelial hyperpermeability by interruption of Src-related signaling [6]. Piegeler and collaborators have shown that ropivacaine and lidocaine inhibit tumor necrosis factor alpha (TNFα)-induced eNOS phosphorylation and subsequent NO production. The mechanism(s) of eNOS inhibition occurred by reducing recruitment of p85 to the TNFα receptor [7].

The observed inhibition of eNOS-mediated signaling by amide LA led us to hypothesize that LA may also interrupt endothelial mechanotransduction and thereby prevent endothelial hyperpermeability.

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https://doi.org/10.1016/j.lfs.2019.02.053
Received 11 October 2018; Received in revised form 18 February 2019; Accepted 25 February 2019
Available online 26 February 2019
0024-3205/

Therefore, we tested the effect of ropivacaine during acute increases in lung capillary pressure on whole lung filtration coefficient (K_f) using the isolated perfused rat lung preparation. Ropivacaine provided robust protection from pressure-dependent barrier failure. We then tested the clinical relevance of ropivacaine on pulmonary barrier function using a rat model of acute hypertension by assessing indices of pulmonary edema, alterations in arterial blood gases and systemic markers of endothelial permeability. We found that ropivacaine provided significant protection against all indices of pressure-dependent endothelial hyperpermeability during acute hypertension. Western blot analysis of whole lung lysates validated that ropivacaine prevents NO production as a primary mechanism for barrier protection. Collectively, the results of this study demonstrate that ropivacaine attenuates pressure-dependent endothelial mechanotransduction and associated barrier failure in both an isolated lung preparation and a whole animal model. Amide LA may be beneficial in reducing pulmonary edema during elevated hydrostatic pressure.

2. Methods

2.1. Reagents

Chemicals were of the highest grade available. Drugs and Krebs-Ringers buffer were purchased from Sigma Chemical (St. Louis, MO); bovine serum albumin was from Proliant Biologicals (Boone, IA); antibodies were purchased from EDM Millipore (Billerica, MA) and Cell Signaling (Danvers MA).

2.2. Isolated perfused lung preparation (IPL)

All animal experiments were approved by the University of Illinois Animal Care Committee. The rat isolated perfused lung preparation was used as previously described [8,9]. Briefly, adult Sprague-Dawley rats (300–400 g) were anesthetized with a mixture of ketamine/xylazine, a tracheotomy was performed, and mechanical ventilation was provided by a pressure-controlled ventilator (Kent Scientific, Torrington, CT) at a respiratory rate of 60/min, peak inspiratory pressure (PIP) of 10 cm H_2O, and positive end-expiratory pressure of 3 cm H_2O. The pulmonary artery and left atria were cannulated and lungs were perfused with Krebs-Ringer bicarbonate solution containing 3% bovine serum albumin. Pulmonary arterial (P_{pa}) and left atrial pressures (P_{LA}) were measured continuously *via* in-line pressure transducers (P-75, Harvard Apparatus, Natick, MA) connected to an analog-to-digital board. An in-line ultrasonic flow probe (Transonic, Ithaca, NY) was placed in line with the pulmonary artery cannula and the lungs were suspended from a force transducer (Radnoti, Minrovia, CA). Flow, vascular pressures, and lung weight were recorded using a custom-written program using Labveiw (National Instruments, Austin, TX). At the end of the protocol, animals were euthanized under general anesthesia *via* exsanguination.

2.3. Calculation of K_f

Pulmonary capillary pressure (P_{pc}) was determined from pulmonary artery pressure (P_{pa}) and left atrial pressure ($P_{LA)}$ using the equation $P_{pc} = (P_{pa} + P_{LA}) / 2$ [10]. The rate of change in lung weight (g) during a two-minute period (18–20 min) following the pressure step was then divided by capillary pressure (P_{pc}) to yield mL/min/cm H_2O [10]. This value was normalized to 100 g of predicted lung weight (PLW) using the equation, $PLW = 0.0053 * (\text{rat weight}) - 0.48$.

2.4. Zonal characteristic (ZC)

K_f is a product of hydraulic conductivity and filtration surface area; thus, changes in vascular recruitment can influence measures of K_f. To determine if ropivacaine altered vascular recruitment, zonal characteristics, *i.e.* percentage of West zone 2 and 3 conditions were derived for each group. Zonal characteristics were calculated according to $ZC = \Delta P_{pa} / \Delta P_{LA}$ as previously described [11,12].

2.5. Experimental protocols

Isolated, perfused lungs were ventilated and kept at isogravimetric pressure for 20 min (P_{LA} = 2 cm H_2O). The P_{LA} was then increased to 7.5 cm H_2O for 20 min. After a second 20-min isogravimetric period the lungs were again subjected to a P_{LA} step to 7.5 cm H_2O (Control), 15 cmH_2O (High P_{LA}), or 15 cm H_2O + Ropivacaine (Ropi + High P_{LA}). Finally, the lungs were returned to isogravimetric condition for a third 20-min period. K_{f1} was derived during the first P_{LA} step; K_{f2} was derived during the second pressure step; pulmonary artery (P_{pa}) and left atrial pressures (P_{LA}) were measured during both pressure steps.

Ropivacaine was added to the perfusate reservoir 10 min prior to the second increase in P_{LA}. The final concentration of ropivacaine was 1 μM.

2.6. Acute hypertension model

Briefly, rats were anesthetized with isoflurane, a tracheotomy was performed and rats were mechanically ventilated on room air (RR = 60/min, PIP = 10 cm H_2O), PEEP = 3 cm H_2O). The left carotid artery and right jugular vein were cannulated for measurement of arterial blood pressure and drug administration, respectively.

Acute hypertension (AH) was induced by a continuous infusion of norepinephrine (starting at 7 μg/kg/min) for 2 h and titrated to maintain a MAP = 150 mm Hg, while control rats (SHAM) only received an infusion of Lactated Ringers buffer (5 mL/kg/h). Ropivacaine was administered as a bolus targeting a plasma concentration of 1 μM before norepinephrine infusion (Ropi + AH). A fourth group of animals received an infusion of lactated ringers buffer (5 mL/kg/h) following the ropivacaine bolus (Ropi). At the end of the acute hypertension protocol, animals were euthanized under general anesthesia *via* exsanguination.

Table 1
Arterial blood gases.

	SHAM	AH	Ropi	Ropi + AH
pH	7.47 ± 0.2	7.23 ± 0.1*	7.48 ± 0.1**	7.40 ± 0.1**
P_aO_2 (mm Hg)	103.7 ± 8.7	67.33 ± 12.7*	101.8 ± 8.4**	97.5 ± 8.7**
P_aCO_2 (mm Hg)	33.33 ± 1.9	34.33 ± 5.9	32.0 ± 2.0	35.33 ± 4.3
HCT	33.5 ± 4.3	40.5 ± 2.3*	34.11 ± 1.9**	40.67 ± 4.8***

Values are mean ± standard deviation. $N \geq 6$/group Abbreviations: P_aO_2 = arterial O_2 pressure (mm Hg); P_aCO_2 = arterial CO_2 pressure (mm Hg); HCT = Hematocrit.

* $p < 0.05$ *vs* SHAM.

** $p < 0.05$ *vs* AH.

*** $p < 0.05$ *vs* SHAM and Ropi.

2.7. Arterial blood gas analysis

Arterial blood gases (ABG) were assessed for pH, P_aO_2, P_aCO_2 and hematocrit (HCT), using a GEM Premier 3000. ABG analysis was repeated every 30 min. Full arterial blood gas results are presented in Table 1.

2.8. Lung wet/dry ratio

Lung samples were collected at the end of the experiment for immediate lung wet weight (LWW) measurement. Samples were placed in an oven set to 60C°. After 24 h, the samples were weighed again for Lung dry weight (LDW). Wet-to-dry (W/D) ratio was determined by the equation: Lung W/D = LWW/LDW.

2.9. Plasma volume loss

During acute hypertension reduction in plasma volume resulted from increased fluid filtration and is an indirect indicator of whole body tissue edema. Changes in plasma volume were calculated based on measurement of hematocrit (HCT) and corrected for total volume of intravenous fluid infusions. The change in plasma volume (%) was determined for each thirty-minute phase of the two-hour experimental period and the % change at 120 min was compared across the groups.

2.10. Assessment of NO production

Mechanotransduction activation was assessed by indirect measurement NO production *via* the quantification of nitro-tyrosine content in whole lung lysates by western blot. Western blots of lung tissue were performed as previously described. Signal was detected by chemiluminescence using Li-COR system and band intensities were measured using Image Studio software (Li-COR, Lincoln, NE).

2.11. Statistical analysis

Data are presented as mean ± SD. Groups were compared using one-way ANOVA or student *t*-test as appropriated. Tukey post-hoc test was used to compensate for multiple test procedures. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Pressure-dependent Increase in whole lung filtration coefficient (K_f)

Baseline K_f (K_{f1}) was determined in all lungs using a step increase in P_{LA} from 3.0 cm H_2O to 7.5 cm H_2O. There were no significant differences in baseline K_{f1} values between the groups and baseline pulmonary artery and capillary pressures were identical between groups. After establishing baseline K_f, Control group (C) was exposed to a second P_{LA} step to 7.5 cmH_2O while the High Pressure group (High P_{LA}) was exposed to a second P_{LA} step = 15 cmH_2O. The K_f measured during the second pressure step was termed K_{f2} and this value was normalized to K_{f1} (K_{f2}/K_{f1}) to determine pressure-dependent changes in K_f [8,9].

Control lungs had a mean $K_{f1} = 0.17 \pm 0.125$ and a $K_{f2} = 0.18 \pm 0.159$ mL/min/cm H_2O per 100 g wet lung weight. Control lungs had a K_{f2}/K_{f1} ratio = 1.13 ± 0.447. In the High P_{LA} group, $K_{f1} = 0.10 \pm 0.051$ mL/min/cm H_2O per 100 g wet lung weight and $K_{f2} = 0.67 \pm 0.385$ mL/min/cm H_2O per 100 g wet lung weight, producing a $K_{f2}/K_{f1} = 6.30 \pm 1.66$, ($p = 0.0002$ *vs* Control). Note that K_{f2} in the High P_{LA} group was about three fold higher than in the control group (0.67 ± 0.385 *vs.* 0.18 ± 0.159, respectively; $p = 0.0319$). The K_{f2}/K_{f1} ratio was 5.3-fold higher in the High P_{LA} group than the control group (Fig. 1A-C). Note that K_f remained stable on control (Fig. 2A) and that the increase on P_{LA} to 15 cm H_2O resulted in a steep increase in K_f as it can be observed as high K_{f2} in the High P_{LA} group (Fig. 2B).

3.2. Ropivacaine attenuates pressure-dependent increases in K_f

Ropivacaine administration (Ropi + High P_{LA} group) attenuated the effects of increased P_{LA} on K_{f2} and K_{f2}/K_{f1}. Baseline $K_{f1} = 0.18 \pm 0.10$ mL/min/cm H_2O per 100 g wet lung weight (Fig. 1A) while ropivacaine reduced K_{f2} to 0.21 ± 0.121 mL/min/cm H_2O per 100 g wet lung weight when compared to High P_{LA} group (0.67 ± 0.385; $p = 0.0397$; Fig. 1B) and reduced K_{f2}/K_{f1} to1.28 ± 0.51 (Ropi + High P_{LA}) (Fig. 1C). K_{f2}/K_{f1} value was significantly lower compared to High P_{LA} alone ($K_{f2}/K_{f1} = 6.30 \pm 1.66$; $p = 0.0001$) but not different from C ($K_{f2}/K_{f1} = 1.14 \pm 0.45$; $p = 0.6488$), indicating that ropivacaine completely attenuated the pressure-dependent increase in K_{f2} (Fig. 1A-C and Fig. 2C).

3.3. Pulmonary hemodynamics

Increase in hydrostatic pressure increased P_{pa} from 12.56 ± 0.44 cm H_2O (Control) to 21.77 ± 2.59 cm H_2O (High P_{LA}) (p = 0.0001) and P_{pc} from 9.78 ± 0.45 cm H_2O to 18.35 ± 1.37 cm H_2O (p = 0.0001) confirming previous reports from this group [9]. All pulmonary hemodynamics during the second pressure step were similar between High P_{LA} *vs.* Ropi + High P_{LA}. There were no differences in pulmonary artery pressure between High P_{LA} (21.77 ± 2.59 cm H_2O) *vs.* Ropi + High P_{LA} (19.93 ± 1.06 cm H_2O, $p = 0.1423$, Fig. 3A). Pulmonary capillary pressure in High P_{LA} (18.35 ± 1.37 cm H_2O) *vs.* Ropi + High P_{LA} (17.48 ± 0.44 cm H_2O) was not statistically significant (Fig. 3B, $p = 0.1739$).

3.4. Zonal characteristics (ZC)

To rule out the effects of ropivacaine on vascular recruitment and vascular surface area that could influence the measurement of K_f, ZC ($\Delta P_{PA}/\Delta P_{LA}$) was derived as an indicator of West zone II and zone III conditions. When P_{LA} = 15 cm H_2O, ZC = 0.76 ± 0.143). Ropi + High P_{LA} had no effect on ZC compared to High P_{LA} alone (0.80 ± 0.125 *vs.* 0.76 ± 0.143; $p = 0.6511$) (Fig. 3C). Therefore, ropi-dependent reduction in K_{f2} does not depend on changes in recruitment and surface area.

3.5. Acute hypertension model

Sham rats demonstrated stable arterial bold gases values over the 120-minute experimental period. Rats in the acute hypertension group demonstrated acute reductions in P_aO_2 from 103.7 ± 8.73 to 67.33 ± 12.69 consistent with pulmonary edema. These reductions resulted in a ΔP_aO_2 of −40 ± 15%, which was significantly different from the ΔP_aO_2 of −7 ± 6% seen in the control group ($p = 0.001$, Fig. 4A). Lung W/D ratio in Shams = 5.23 ± 0.46 while in acute hypertension group = 6.44 ± 0.75, consistent with increased pulmonary edema (Fig. 4B). Arterial blood gases for sham and acute hypertension rats are found in Table 1.

3.6. Ropivacaine attenuates pulmonary edema

Ropivacaine-treated rats had no change in arterial blood gas values when compared to sham rats (Table 1). Ropivacaine-treated rats subjected to AH (Ropi + AH) maintained similar MAP throughout the length of the study compared to AH alone. However, ropivacaine reduced the magnitude of pulmonary edema during acute hypertension. Ropivacaine treatment significantly prevented the decrease in P_aO_2 (Ropi + AH = 15% reduction *vs.* AH = 40% reduction, $p = 0.0022$) and ropivacaine-treated rats had a lower lung W/D ratio (Ropi + AH = 4.80 ± 0.18 *vs.* AH = 6.44 ± 0.75, $p = 0.0004$) (Table 1; Fig. 4A and B, respectively). Ropivacaine also reduced the loss

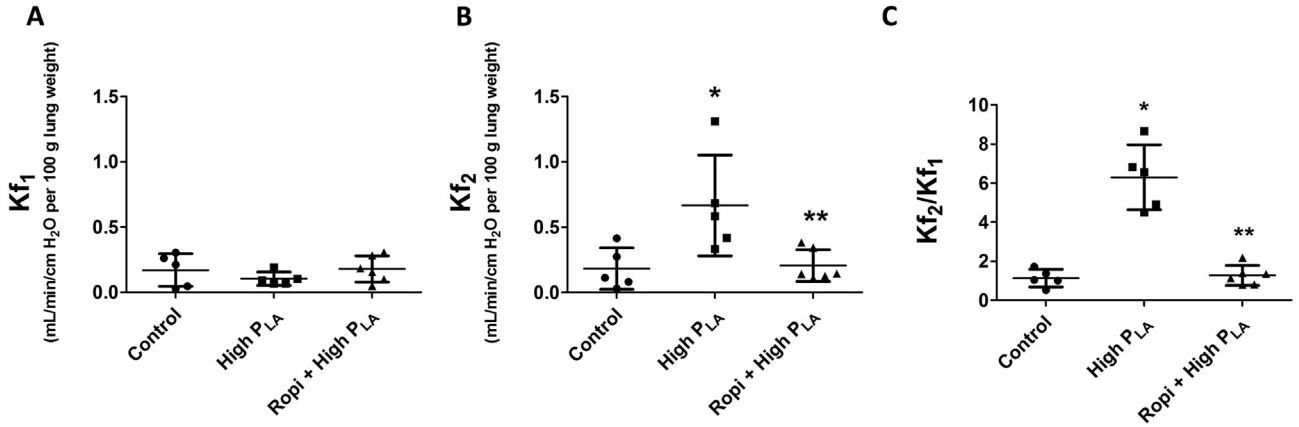

Fig. 1. Effect of Ropivacaine on Pressure-Dependent Increases in K_f. A. Baseline K_{f1} is shown for all groups. Groups are as follows: Control, high pressure (High PLA) and ropivacaine + high pressure (Ropi + high PLA). A. Note that starting K_f are similar for all groups. B. K_{f2} for all groups. Note that ropivacaine completely attenuates the increase in K_{f2} during high pressure (Ropi + High PLA). C. Ratio of K_{f2}/K_{f1} increased over 5-fold ($p < 0.0018$) during high pressure (High PLA) and was completely attenuated by ropivacaine (Ropi + High PLA; $p < 0.0001$). $N \geq 5$/group. *$p < 0.05$ *vs* Control; ** $p < 0.05$ *vs* High P_{LA}.

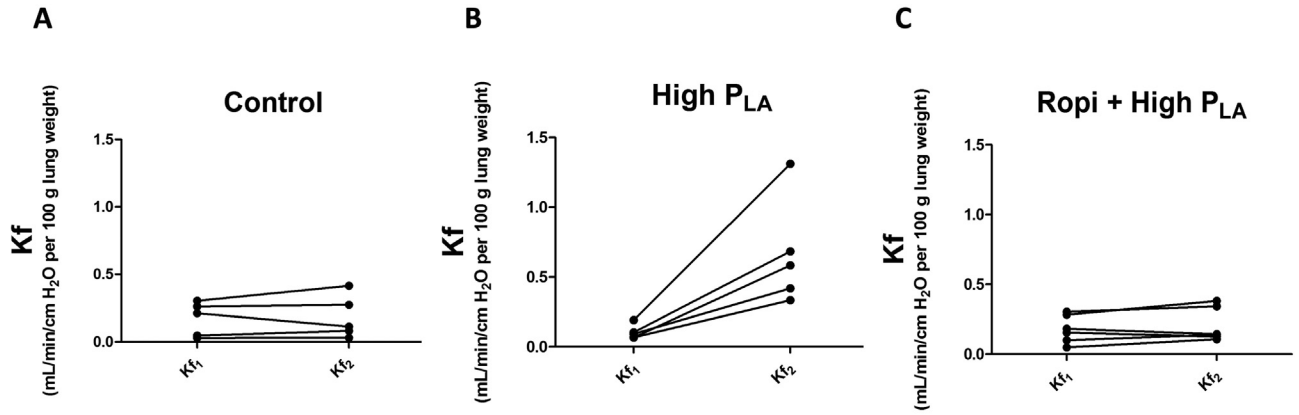

Fig. 2. Changes in K_f with pressure and ropivacaine. To highlight the changes in K_f with changes in pressure (High P_{LA}) and ropivacaine (Ropi + High P_{LA}), we present the individual data for each experiment to demonstrate both the magnitude and consistency of the K_f response. A. Control lungs were exposed to two pressure steps where P_{LA} = 7. 5 cm H_2O. K_{f1} and K_{f2} were not altered. B. High P_{LA} were exposed to P_{LA} = 7.5 cm H_2O and then 15 cm H_2O which resulted in an increase in Kf and thus, higher K_{f2}. C. Ropivacaine treatment during high pressure (Ropi + High P_{LA}) completely prevented the pressure-dependent increase in K_f and maintained K_f at control levels. N = 5/group.

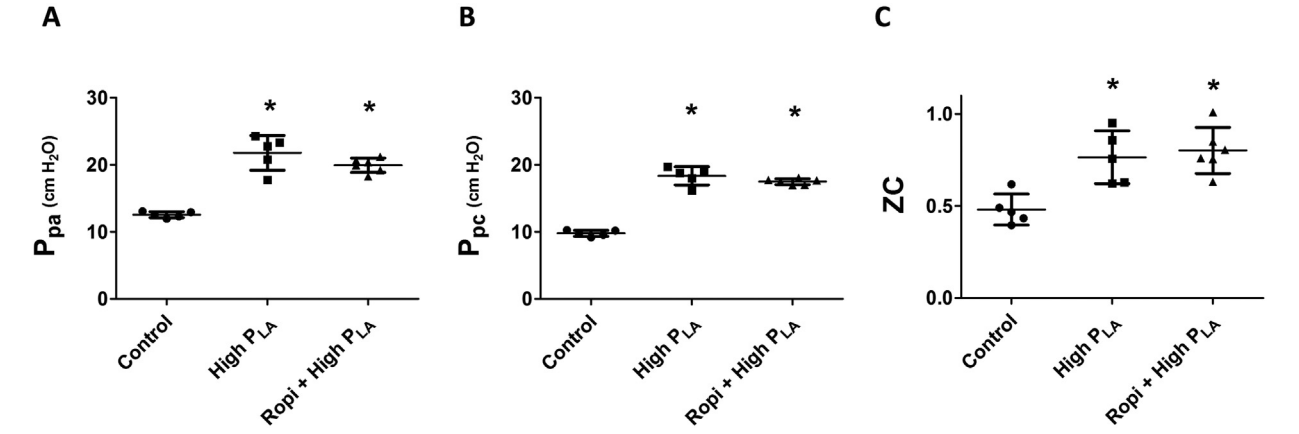

Fig. 3. Pulmonary hemodynamics. The effect of High pressure (High P_{LA}) and ropivacaine (Ropi + High P_{LA}) on pulmonary artery (P_{pa}) and pulmonary capillary pressures (P_{pc}) were compared. A. An increase in P_{LA} caused an increase in P_{pa}, thus the High P_{LA} group had higher P_{pa} compared to Control. Ropivacaine treatment (Ropi + High P_{LA}) had no effect on P_{pa} compared to High P_{LA} lungs (19.93 ± 1.06 *vs.* 21.77 ± 2.59 cm H_2O; (p = 0.1423). B. Pulmonary Capillary Pressure. Ropivacaine treatment (Ropi + High P_{LA}) had no effect on capillary pressure compared to High P_{LA} lungs (17.48 ± 0.47 *vs.* 18.35 ± 1.37 cm H_2O), (p = 0.1739) C. Zonal Characteristics Ropivacaine (Ropi + High P_{LA}) had no effect on Zonal Characteristics (ZC). Ropivacaine-treated lung had a ZC = 0.76 ± 0.08 while High P_{LA} had ZC = 0.80 ± 0.12. $N \geq 5$/group. *$p < 0.05$ *vs* Control.

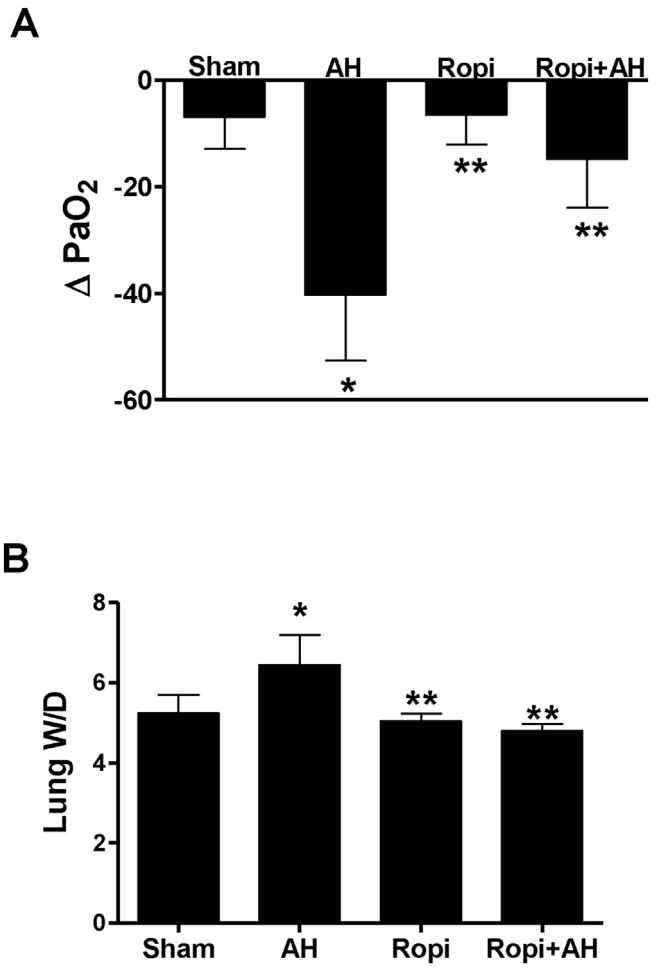

Fig. 4. Effect of ropivacaine on pulmonary edema. A. Arterial Oxygen Partial Pressure. Acute hypertension (AH) caused a rapid reduction in arterial partial pressure (ΔP_aO_2) to −40 ± 15% of Controls (Sham). Ropivacaine alone (Ropi) had no effect on ΔP_aO_2 relative to sham (−7 ± 6%). Ropi + AH significantly protected lungs as ΔP_aO_2 fell only −15 ± 9% ($p = 0.0022$). B. Change in Lung Wet-Dry Ratio. Sham lungs had a W/D = 5.23 ± 0.46 while lungs from AH group had an increased W/D to 6.44 ± 0.75 (23% increase; $p = 0.0073$ *vs.* Control). Ropivacaine alone (Ropi) had no effect on W/D (5.04 + 0.19) but significantly reduced lung W/D during AH (Ropi + AH; 4.80 ± 0.18, $p = 0.0004$). $N = 6$/group. *$p < 0.05$ *vs* SHAM; **$p < 0.05$ *vs* AH.

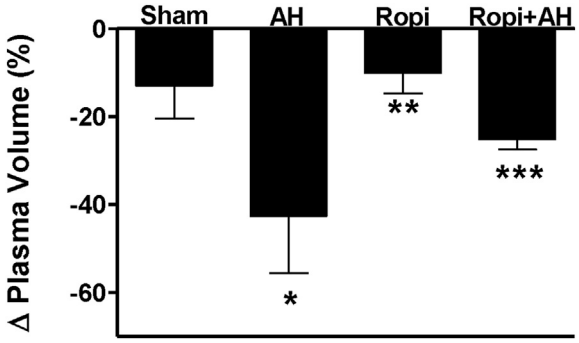

Fig. 5. Effect of ropivacaine on plasma volume loss. Sham rats lost an average of 13 ± 7% in plasma volume over 120 min; hypertensive rats (AH) experienced a 43 ± 13% ($p = 0.0007$) reduction in plasma volume. Ropivacaine alone (Ropi) had no effect on plasma volume relative to Sham (10 ± 5%) but Ropivacaine + Acute Hypertension (Ropi + AH) had a significantly reduce loss in plasma volume (25 ± 2%) compared to the AH group ($p = 0.009$). N ≥ 5/group. *$p < 0.05$ *vs* SHAM; **$p < 0.05$ *vs* AH; *** $p < 0.05$ *vs* Ropi.

in plasma volume during the acute hypertensive state. Acute Hypertension rats demonstrated a 43% loss in plasma volume while Ropi + AH group lost only 25% ($p = 0.0090$) (Fig. 5). Lastly, as an additional control measure, a separate group of rats were given a single-bolus of ropivacaine as the sole treatment and were maintained at normotensive blood pressure (Ropi); there were no statistical differences between this group and control (Sham) for any of the measured arterial blood gases (Table 1) and changes in plasma volume (Fig. 5).

3.7. Ropivacaine inhibits NO production during acute hypertension

Pressure-dependent NO production is a key mediator of endothelial hyperpermeability [3,13,14], [8] such that increased NO production is a marker for endothelial mechanotransduction. To determine if ropivacaine inhibited hypertension-induced pulmonary edema by inhibiting NO production, we indirectly assessed NO generation by quantifying nitration of tyrosine residues in lung lysates. As shown in Fig. 6, while hypertensive lungs showed an increase in nitro-tyrosine lung content, confirming our previous findings, lungs from the Ropi + AH group were no different when compared to sham rat lungs, indicating that ropivacaine inhibited hypertension-dependent NO formation (Fig. 6).

4. Discussion

We assessed the effect of ropivacaine, an amide local anesthetic, on pressure-dependent endothelial barrier failure using two models that simulate the pulmonary hemodynamics of acute severe hypertension. Ropivacaine completely prevented pressure-dependent increase in K_f in the IPL model and attenuated indices of pulmonary edema in a rat model of severe hypertension. Ropivacaine also demonstrated protection against plasma volume loss, an indirect measure of systemic fluid filtration, suggesting that it protective effects were not confined to the lungs. Western blot analysis of whole lung lysates demonstrated that ropivacaine significantly reduced the amount of nitrated-tyrosine residues on lung proteins, consistent with our previous work on LA-mediated inhibition of eNOS [6]. Collectively, these results validate previous work from our laboratory demonstrating pressure-activated eNOS-dependent mechanism that results in endothelial hyperpermeability. This new finding demonstrates that ropivacaine attenuates endothelial mechanotransduction in a rat model of acute severe hypertension and suggests LA may have a role in preventing and treating pulmonary edema associated with increased pulmonary vascular pressure.

In the isolated perfused rat lung preparation, doubling of left atrial pressure from 7.5 to 15 cm H_2O produced an increase in capillary pressure from 9.8 to 18.3 cm H_2O, that resulted in a 5-fold increase in the filtration co-efficient (K_f) in only 20 min. This is consistent with previous findings from this laboratory [3,8]. We recognize that at baseline $P_{LA} = 7.5$ cm H_2O, the lung vasculature is not fully recruited (ZC = 0.48) and K_f is underestimated. When $P_{LA} = 15$ cm H_2O, ZC approaches 0.80 which is a nearly fully recruited lung and K_f is an accurate representation of the filtration. Ropivacaine had no effect on ZC or pulmonary vascular hemodynamics at high pressure yet resulted in a significant reduction in K_{f2}. These data strongly suggest that ropivacaine reduced endothelial permeability.

We then tested the ability of ropivacaine to attenuate pulmonary edema in a model of acute severe hypertension created by increasing afterload, LVEDP and pulmonary capillary pressure [15,16,18]. Ropivacaine administration prior to acute hypertension significantly prevented the deterioration in P_aO_2 and lung wet/dry ratio. These results support the findings from the IPL data, suggesting the ropivacaine prevents pressure-dependent endothelial hyperpermeability in an intact rat model. Ropivacaine reduced the amount of nitro-tyrosine residues on lung proteins supporting our hypothesis that its barrier-protective effects are mediated by preventing NO production.

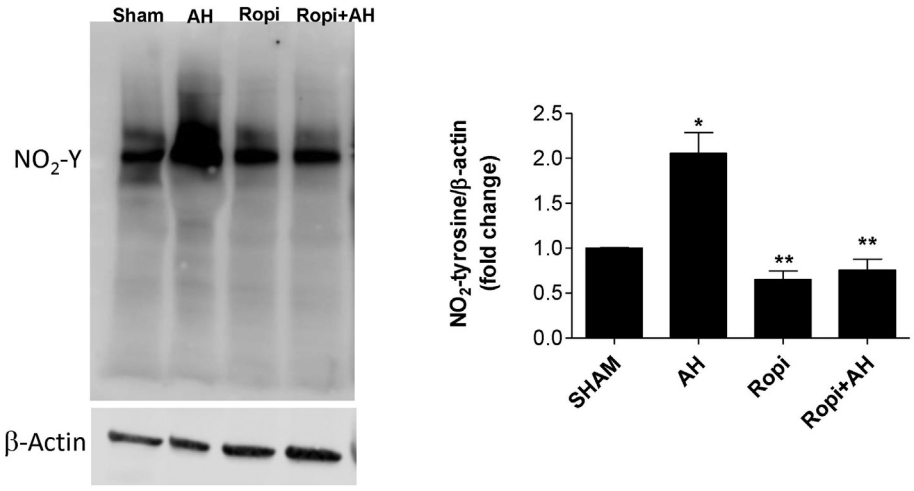

Fig. 6. Effect of ropivacaine on nitro-tyrosine in whole lung lysates. We assessed the increase in total nitro-tyrosine (NO_2-Y) residues from whole lung lysates as a measure of increased NO production. Acute hypertension (AH) resulted in a 2-fold increase in nitro-tyrosine immunolabeling relative to sham lungs. Ropivacaine alone (Ropi) had no effect of nitro-tyrosine content but ropivacaine returned nitro-tyrosine content in hypertensive lungs to sham levels (Ropi + AH). $N \geq 4$/group. $^{*}p < 0.05$ *vs* SHAM; $^{**}p < 0.05$ *vs* AH.

Although our focus was on lung endothelial mechanotransduction, there is evidence that the acute hypertension also caused barrier failure throughout the circulation. We noted that HCT continually increased during the 2-hour period of acute hypertension. We used the change in HCT to derive a change in plasma volume over the 120-minute experimental period. Hypertensive rats had a 43% reduction in total plasma volume while ropivacaine administration reduced plasma volume loss to 25%. This observation suggests that ropivacaine stabilized barrier function throughout the vascular system.

4.1. Clinical relevance

These data suggest that ropivacaine may be a clinically useful agent to prevent increases in endothelial permeability when pulmonary hydrostatic pressure is acutely increased. Clinical examples of these conditions include hypertensive emergencies; acute heart failure; following lung resection surgeries, especially pneumonectomy, when the remaining lung must receive the total cardiac output; and neurogenic pulmonary edema.

The concentrations of LA used in this study are clinically relevant. Perotti and collaborators [17] measured plasma concentrations of ropivacaine in 85 patients receiving a continuous infusion *via* a thoracic epidural catheter and reported values ranging from 0.22 to 4.85 μg/mL. The concentration of ropivacaine used in the IPL model (1 μM = 0.367 μg/mL) represents a clinically relevant plasma concentration obtained after 48 h of thoracic epidural infusion. In fact, 1 μM is at the very low end of this range yet was still capable of completely preventing the pressure-dependent increase in K_f in the IPL. In the rat acute hypertension model, we intentionally choose a low dosing scheme to prevent cardio-toxic effects. The bolus dose of ropivacaine was calculated to achieve an acute plasma concentration of 1 μM without accounting for clearance and without using allometric scaling. Despite the low plasma concentration, ropivacaine administration prior to inducing hypertension was able to significantly prevent the drop in P_aO_2 and the increase in W/D.

5. Conclusion

Endothelial hyperpermeability is a primary mechanism for development of pulmonary edema, a severe life-threatening condition that may occur secondary to hypertensive emergencies; acute heart failure and following lung resection surgeries. Our data indicate that ropivacaine may be a clinically useful agent to prevent endothelial hyperpermeability and pulmonary edema development.

Ethics approval and consent to participate

All animal experiments were approved by the University of Illinois Animal Care Committee.

Competing interests

None to declare.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

MP wrote the manuscript, performed experiments, data analysis and interpretation.

AZC wrote the manuscript, contributed to study design, performed experiments, data analysis and interpretation.

NB performed experiments and assisted on data analysis.

AI performed experiments.

ROD was responsible for study design, wrote the manuscript, supervised experiments and data analysis and interpretation.

Acknowledgements

Not applicable.

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