Research Article

Differences between basal lung levels of select eicosanoids in rat and mouse

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ABSTRACT

Metabolites of arachidonic acid play an important role in mediating inflammation, cell proliferation, and oxidative stress that contribute to many pulmonary diseases. We hypothesized that the substantial differences between rats and mice in their responses to experimental pulmonary hypertensive stimuli would be associated with parallel differences in their basal eicosanoid profile. Rat and mouse lung extracts were subjected to liquid chromatography tandem mass spectrometry that was optimized for simultaneous separation and rapid quantification of the major hydroxyeicosatetraenoic acids (HETEs) and prostaglandins (PGs). Basal levels (pg/μg protein) of arachidonic acid metabolites differed significantly between rat and mouse lungs. Median values of the following major eicosanoids were significantly higher in mouse than in rat lungs: 5-HETE, 8-HETE, 12-HETE, 15-HETE, PGE_2, and PGI_2, as well as isoprostane-E_2 and -$F_{2\alpha}$. In addition, the PGI_2/TXB_2 ratio was increased in mouse relative to rat lungs. On the basis of the important roles that these compounds play in determining pulmonary vascular tone, the differences in select eicosanoid profiles, especially the PGI_2/TXB_2 ratio, between rat and mouse lungs may underlie the interspecies differences in susceptibility to the development of pulmonary hypertension.

Key Words: eicosanoids, LC/MS/MS, lipoxygenase, liquid chromatography, prostacyclin, prostaglandin, pulmonary hypertension, tandem mass spectrometry, thromboxane

The lung vasculature synthesizes various eicosanoids derived from arachidonic acid. The conversion of arachidonic acid into many metabolites is achieved in a highly regulated manner, and many arachidonic acid derivatives have been linked to various disease states. Hydroxyeicosatetraenoic acids (HETEs) are produced enzymatically from arachidonic acid and the principal mammalian enzymes for arachidonic acid metabolism are lipoxygenases (LOX), cytochrome P450 isoenzymes and cyclooxygenase.[1] Three major LOXs (5-, 12-, and 15-LOX) have been identified in various tissues[1] and several reports suggest that they participate in vascular remodeling in pulmonary hypertension.[2-4]

Similar to HETEs, prostaglandins (PGs) are products that are enzymatically derived from arachidonic acid and act as autocrine and paracrine effectors to regulate pulmonary vascular tone. The major PG generated by the endothelium of pulmonary arteries is prostacyclin (PGI_2), a potent vasodilator which inhibits platelet aggregation and has antiproliferative and anti-inflammatory properties.[5] Conversely, thromboxane A_2 (TXA_2), produced from arachidonic acid via the action of thromboxane synthase, opposes the actions of PGI_2 by promoting vasoconstriction, platelet aggregation, and smooth muscle cell proliferation.[6]

Decreased local production and availability of PGI_2 have been implicated in the pathogenesis of pulmonary hypertension. Using a rat model, Rabinovitch et al.[7] showed that induction of PGI_2 release prevents hypoxic pulmonary hypertension and vascular remodeling. In addition, translational research showed that patients with

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pulmonary arterial hypertension (PAH) have an abnormally low ratio of urinary PGI_2 to TXA_2 metabolites, and this imbalance is thought to participate in the pulmonary vasoconstriction and local thrombosis that characterizes this condition.[8-9]

The importance of lipid mediators in modulating inflammation, cell proliferation, and pulmonary vascular remodeling is highlighted by the therapeutic use of various modulators of these pathways, with resultant amelioration of disease states. Replacement therapy with PGI_2[10] or its analogues[11] is being used in the treatment of pulmonary hypertension. Therefore, there has been an ongoing interest in understanding the contribution of various lipid metabolites to the pathophysiology of pulmonary vascular disorders.

Experimental pulmonary hypertension studies in rodents have revealed remarkable interspecies differences. When exposed to hypoxia or monocrotaline, rats develop more severe pulmonary hypertension than mice.[12] Pulmonary vascular remodeling, inflammation, and pressure elevation are all more pronounced in rats, but the mechanisms explaining the interspecies differences have not been elucidated. Because of the evidence suggesting a role for bioactive lipids in the pathogenesis of PAH, we hypothesized that rat and mouse lungs would have different eicosanoid profiles associated with their differing pulmonary hypertensive responses.

MATERIALS AND METHODS

Materials

The following compounds were purchased from Cayman Chemical Co. (Ann Arbor, Mich., USA): 5-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid (5-HETE); 8-hydroxy-5Z,9E,11Z,14Z- eicosatetraenoic acid (8-HETE); 12-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid(12-HETE); 15-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid(15-HETE); 9-oxo-11R,15S-dihydroxy-5Z,13E- prostadienoic acid (PGE_2); 9-oxo-11S,15S-dihydroxy-5Z,13E- prostadienoic acid(11β-PGE_2); 9,15-dioxo-11R-hydroxy- 5Z,13E-prostadienoic acid (15-keto-PGE_2); 9,15-dioxo-11R-hydroxy-5Z-prostenoic acid(13,14-dihydro-15-keto-PGE_2); 9S,15S-dihydroxy-11-oxo-5Z,13E-prostadienoic acid (PGD_2); 11,15-dioxo-9S-hydroxy-5Z-prostenoic acid (13,14-dihydro-15-keto-PGD_2); 9S,11R,15S-trihydroxy-5Z,13E-prostadienoic acid ($PGF_{2\alpha}$); 9S,11S,15S-trihydroxy-5Z,13E-prostadienoic acid(11β-$PGF_{2\alpha}$); 6-oxo-9S,11R,15S- trihydroxy-13E-prostenoic acid(6-keto-$PGF_{1\alpha}$); 9S,11,15S- trihydroxy-thromboxa-5Z,13E-dien-1-oic acid (TXB_2); 9-oxo-11R,15S-dihydroxy-5Z,13E-prostadienoic acid-cyclo[8S,12R] (8-iso-PGE_2), 9S, 11S, 15S- trihydroxy-(8β)- prosta-5Z, 13E-dien-1-oic acid(8-iso-$PGF_{2\alpha}$); 9S,11R-dihydroxy-15-oxo-5Z-prostaenoic acid-cyclo [8S,12R] (8-iso-13,14-dihydro-15-keto-$PGF_{2\alpha}$).

Internal standards mixture (Cayman Chemical Co., Ann Arbor, Mich., USA): 5S-hydroxy-6E,8Z,11Z,14Z- eicosatetraenoic acid (5,6,8,9,11,12,14,15-d8) [5(S)-HETE-d_8]; 12S- hydroxy-5Z,8Z, 10E, 14Z- eicosatetraenoic acid-d8 [12(S)- HETE-d_8]; 15S-hydroxy-5Z,8Z,11Z,13E- eicosatetraenoic acid-d8 [15(S)-HETE-d_8]; 11R,15S-dihydroxy-9-oxo-5Z,13E-prostadienoic acid(3,3,4,4-d4)(PGE_2-d_4); 9S,11,15S-trihydroxy-thromboxa-5Z,13E-dien-1-oic acid-d4 (TXB_2-d_4). Gradient grade methanol, water, hexane, and ammonium acetate for high-performance liquid chromatography (HPLC) ≥99.9% were purchased from Sigma Aldrich (St. Louis, MO).

Animals

Animals were purchased from Taconic, Germantown, N.Y., USA. Male mice (C 57 BL/6, 30-35 g) and rats (Sprague Dawley, 250-300 g) were maintained on standard laboratory chow diet and water ad libitum. After animals were euthanized with a pentobarbital injection (120 mg/kg ip), the thorax was opened immediately, the lungs were removed, weighed, frozen in liquid nitrogen, and stored at −80°C. The ages of the animals used for this study were between two and four months. The protocol was approved by the IACUC at Tufts Medical Center and was conducted in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

Lung sample extraction and preparation

Lipid extraction of lung tissue was carried out as previously described, with modifications.[13,14] Briefly, lung tissue was homogenized for 30 seconds in 2 mL in 66% methanol. After 100 μL were removed for protein quantification, the samples were frozen in -80°C for 45 minutes for protein precipitation. The homogenates were then centrifuged at 4,500 rpm for 30 minutes at 4°C. The supernatant was diluted ≥ 10-fold with ice-cold HPLC grade water. The diluted supernatant was acidified on ice to a pH of 4.0 with 1N HCl. Using an Agilent 25 μL glass syringe, a 20μL aliquot of internal standard master mix (5-, 12-, 15-, HETE-d_8, PGE_2-d_4, TXB_2-d_4) was added to each sample (total of 2 ng). The acidified supernatant containing the internal standard was loaded onto an Agilent C18 6 mL, 500 mg cartridge that was conditioned with 14 mL methanol, 14 mL water by low feeding speed in sequence. Following the addition of the sample, the cartridge was washed with 14 mL of HPLC grade water to remove any unabsorbed material and with 14 mL HPLC grade ice-cold hexane to remove any interfering lipids. The analytes were collected in glass Pyrex tubes by elution with 7 mL HPLC grade methanol. The

solvent was evaporated under a gentle stream of nitrogen. The residue was reconstituted with 100 μL mobile phase, vortexed briefly and transferred to an autosampler vial insert for LC/MS/MS analysis. All extraction procedures were performed under minimum light conditions to avoid photodegradation of arachidonic acid metabolites. Protein concentrations were determined using the Bradford assay.[15]

HPLC simultaneous separation of arachidonic acid metabolites

A Phenomenex Luna 5 micron C18 (Phenomenex, Torrance, CA; 150 × 2.00 mm × 5 μm) was used for HPLC. The Agilent 1200 Series HPLC system consisted of a high-performance binary pump, a 108 well plate autosampler at 4°C, and column compartment set at 25°C.The solvent system consisted of 100% methanol and 10 mM ammonium acetate, pH 8.5. Prior to each run, the analytical column was pre-equilibrated with methanol for 60 seconds, and the injection syringe was washed for eight seconds in the flush port. A total of 25 μL of the sample was drawn up at a speed of 200 μL/min with a 100 μl syringe and was ejected onto the column at a rate of 400 μL/min. The flow rate of solvent through the column was 400 μL/min. The analytical column was primed with 100% ammonium acetate for 0.11 minutes and immediately switched to 50% methanol and 50% ammonium acetate at five minutes. At five minutes, methanol was programmed to increase linearly from 50% to 100% over a period of five minutes, after which the flow remained at 100% methanol for 20 minutes to ensure elution of all analytes. At 30.10 minutes, the flow immediately switched to 100% ammonium acetate for the remainder of the 35 minutes run, to flush the methanol entirely from the column. In order to get a complete separation of HETEs and PGs in the same run and under the same conditions, the column was flushed with 100% ammonium acetate for the last 4.90 minutes of each run.

MS/MS targeted profiling of hydroxyeicosatetraenoic acids and prostaglandins

Targeted profiling of HETEs and PGs was performed using a 5500 QTRAP (ABSciex) hybrid triple quadrupole linear ion trap mass spectrometer equipped with a turbo ion-spray ionization source.[16] Multiple reaction monitoring (MRM) experiments were performed using negative ion- spray mode. Detection of eicosanoids was separated into two periods and the dwell time used for MRM experiments was 400 msec for HETEs and 120 msec for PGs and TXB_2, respectively. The collision exit potential was –11 V for HETEs and –13 V for PGs and TXB_2. The declustering potential was –250 V for HETEs and –150 V for PGs and TXB_2. The source-dependent MS parameters such as temperature and ion-spray voltage were set at 350°C and –4500 V, respectively. Collision energy was determined for each specific compound. Using a valco valve diverter, analytes were sent to waste for the first eight minutes of the run and then sent to the MS/MS where they were collected for 22 minutes of the run and then diverted back to waste for the remainder of the run. For details, see Tables 1S and 2S in supplemental data.

Statistical analysis

Values are reported in pg/μg protein, as median (25-75% confidence interval), or mean ± SD. Statistical analysis was performed using Sigma Stat 3.1 statistical software. Student's *t*-test or Wilcoxon-Mann-Whitney-U test was used to determine statistical significance when the normality passed or failed, respectively. $P < 0.05$ was considered statistically significant.

Table 1S: MRM specifications for measurement of select hydroxyeicosatetraenoic acids

HETEs	Specific MRM Transition (*M/Z*)	CE (V)
5-HETE-d_8	327.4 → 116.1	-20.18
5-HETE	319.4 → 115.1	-20.18
8-HETE	319.4 → 155.1	-20.0
12-HETE-d_8	327.4 → 184.1	-20.0
12-HETE	319.4 → 179.1	-20.0
15-HETE-d_8	327.4 → 226.2	-21.0
15-HETE	319.4 → 219.2	-21.0

Transition masses were determined by MS/MS, and are specific to each analyte. Each HETE, with the exception of 8-HETE, had a deuterated internal standard, used for normalization and quantification. 8-HETE was normalized to 12-HETE-d_8 internal standard. **HETE:** hydroxyeicosatetraenoic acid; MRM, multiple reaction monitoring; **CE:** collision energy; **CXP:** collision exit potential; **DP:** declustering potential; **V:** volts

Table 2S: MRM specifications for measurement of select prostaglandins and thromboxane

Eicosanoids	Specific MRM Transition (*M/Z*)	CE (V)
PGE_2-d_4	355.2 → 193.1	-21.0
PGE_2	189.0 → 120.0	-21.0
15-keto-PGE_2	349.4 → 235.1	-22.0
8-iso-PGE_2	189.0 → 120.0	-21.0
11β-PGE_2	189.0 → 120.0	-21.0
13,14-dihydro-15-keto-PGE_2	351.4 → 174.9	-27.77
PGD_2	351.4 → 233.2	-14.0
13,14-dihydro-15-keto-PGD_2	351.4 → 175.0	-29.2
8-iso-$PGF_{2\alpha}$	353.4 → 309.3	-24.42
$PGF_{2\alpha}$	353.4 → 309.3	-24.42
11β-$PGF_{2\alpha}$	353.4 → 193.0	-32.0
8-iso-13,14-dihydro-15-keto-$PGF_{2\alpha}$	353.4 → 183.0	-33.71
6-keto-$PGF_{1\alpha}$	369.4 → 245.1	-35.71
2,3-dinor-6-keto-$PGF_{1\alpha}$	341.4 → 243.0	-35.71
TXB_2-d_4	373.4 → 173.1	-34.00
TXB_2	369.2 → 169.2	-29.00

Transitions selected were specific for each analyte, producing optimal sensitivity for analysis. Analytes with the same transition specifications, such as 8-iso-PGE_2, PGE_2, and 11β-PGE_2 on one hand, and 8-iso-$PGF_{2\alpha}$, $PGF_{2\alpha}$ and 11β-$PGF_{2\alpha}$ on the other hand, eluted at different times, and were separated and identified based on retention time. PGs analytes were normalized to PGE_2-d_4 internal standard and corrected for variations in sensitivity and TXB_2 was normalized to TXB_2-d_4 internal standard. **MRM:** multiple reaction monitoring; **CE:** collision energy.

RESULTS

Lipid extraction from rodent lungs

In order to ensure feasibility of comparison between species, lipid extraction was carried out under the same protocol for both rat and mouse lungs. Extraction efficiencies were 70.3 ± 17.0% for rat lung samples and 68.1 ± 26% (mean ± SD) for mouse lung samples. For detailed information on extraction efficiencies of various eicosanoids, see supplemental data (Table 3S).

Table 3S: Extraction efficiency for select lipid metabolites in rodent lung tissue

Internal Standard	Mouse Lung (N=10)	Rat Lung (N=10)
5-HETE-d_8	71±9	62 ± 22
12-HETE-d_8	71±10	80 ± 42
15-HETE-d_8	73±23	66 ± 15
PGE_2-d_4	92±11	84 ± 37
TXB_2-d_4	61±15	56 ± 10

Extraction efficiencies are reported as mean ± standard deviation of % recovery for each analyte. There were no significant differences between species in extraction efficiencies for the arachidonic acid metabolites. For rest of abbreviations, see Tables 1S and 2S.

Table 1: Concentrations of select hydroxyeicosatetraenoic acids in rodent lung tissue

Analyte	Rat (*N*=10)	Mouse (*N*=10)	*P* value
5-HETE	0.019 (0.009, 0.021)	0.035 (0.022, 0.054)	0.026
8-HETE	0.039 (0.02, 0.053)	0.48 (0.38, 0.59)	<0.001
12-HETE	0.37 (0.277, 0.464)	31.36 (23.15, 41.01)	<0.001
15-HETE	0.040 (0.023, 0.073)	0.336 (0.272, 0.358)	<0.001

Concentrations of analytes (pg/μg protein) are reported as median (25-75% confidence interval). There were significant differences between rat and lung tissue in all analytes detected. **HETE:** hydroxyeicosatetraenoic acid

Differences in HETEs between rats and mice

Analysis of lung samples from 10 rats and 10 mice showed significant interspecies differences. Basal levels of 5-, 8-, 12-, and 15-HETEs were detected in all samples from both rat and mouse lungs. MS/MS fragmentation characteristics are presented in Table 1S. While there was overlap in the values of various HETEs between species, mouse lungs had higher concentrations of all compounds measured (Fig. 1, Table 1). Median values of the 5-, 8-, and 15-HETE were approximately two to 12 times higher in mouse than in rat lungs. The major HETE in both species was 12-HETE, whose levels were 80-fold higher in mouse samples.

Differences in prostaglandins, isoprostanes, and thromboxane between rat and mouse lungs

Based on characteristic MS/MS fragmentations (Table 2S) and retention times for different compounds, we identified the following compounds and their corresponding metabolites and isomers: PGE_2 (with one isomer and two metabolites); PGD_2 (with one metabolite); $PGF_{2\alpha}$ (with one isomer); two PGI_2 metabolites, one thromboxane metabolite (TXB_2), and three compounds of the isoprostane pathway (Tables 2 and 3). Many PGs were nondetectable in the rat compared to mouse lungs [Table 2]. Among the compounds that were detectable in both species, PGI_2 metabolites were significantly higher in mouse than in rat

Table 2: Concentrations of select prostaglandins and thromboxane in rodent lung

PGs	Rat (*N*=10)	Mouse (*N*=10)	*P* value
PGE_2	0.03 (0.02, 0.03)	0.4 (0.3, 0.5)	0.003
11β-PGE_2	0.07 (0.00, 0.1)	0.3 (0.2, 0.3)	0.014
15-keto-PGE_2	ND	0.029 (0.02, 0.06)	<0.001
13,14-dihydro-15-keto-PGE_2	ND	0.08 (0.065, 0.097)	<0.001
PGD_2	ND	0.09 (0.03,0.2)	<0.001
13,14-dihydro-15-keto-PGD_2	ND	0.095 (0.07, 0.1)	<0.001
$PGF_2\alpha$	ND	0.09 (0.08, 0.12)	<0.001
11β-$PGF_2\alpha$	ND	0.046 (0.043, 0.07)	<0.001
6-keto-$PGF_{1\alpha}$	0.036 (0.00, 0.206)	0.72 (0.67, 0.93)	<0.001
2,3-dinor-6-keto-$PGF_{1\alpha}$	ND	0.214 (0.132, 0.27)	<0.001
Sum of PGI_2 metabolites	0.036 (0.00, 0.206)	1.002 (0.35,0.11)	<0.001
TXB_2	0.13 (0.087, 0.20)	0.20 (0.16, 0.24)	NS
PGI_2/TXB_2	0.327 (0.00, 1.29)	4.96 (2.6, 6.4)	<0.001

Concentrations of select analytes (pg/μg protein) are reported as median (25-75% confidence interval). Parent compounds and their 11β-isomers are marked in bold. Analytes in the -keto-, -dinor-, or -dihydro-forms are metabolites of their parent ion. Only TXB_2 had similar levels in both species. **PG:** prostaglandin; **PGI_2:** prostacyclin; **TXB_2:** thromboxane B_2

Table 3: Concentrations of select isoprostanes in rodent lung

Isoprostanes	Rat (*N*=10)	Mouse (*N*=10)	*P* value
8-iso-PGE_2 (isoprostane-E_2)	0.0003 (0.0002, 0.0007)	0.004 (0.003, 0.004)	0.003
8-iso-$PGF_{2\alpha}$ (isoprostane-$F_{2\alpha}$)	ND	0.02 (0.017, 0.023)	<0.001
8-iso-13,14-dihydro-15-keto-$PGF_{2\alpha}$	ND	ND	

Concentrations of select isoprostanes (pg/μg protein) are reported as median (25-75% confidence interval). Isoprostane-E_2 was the only compound detected in rat lungs and it was found in very low concentrations. Mouse lungs had detectable levels of both isoprostane-E_2 and -$F_{2\alpha}$. For abbreviations see Table 2

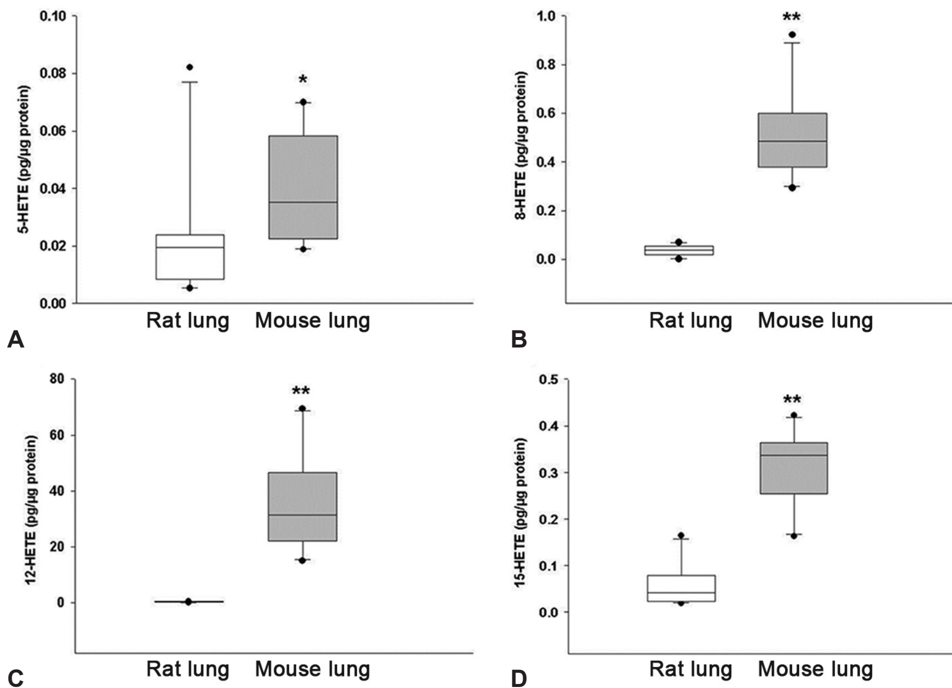

Figure 1: Differences in select hydroxyeicosatetraenoic acids (HETEs) between rat and mouse lungs. Concentrations of 5-HETE **(A)**, 8-HETE **(B)**, 12-HETE **(C)**, and 15-HETE **(D)** are reported. * $P < 0.05$, ** $P < 0.001$

lungs and TXB_2, the TXA_2 metabolite had similar levels. Accordingly, there was a significantly higher PGI_2/TXB_2 ratio in mouse than in rat lungs (Fig. 2). Lastly, PGE_2, a bronchodilator and vasodilator,[17] was also found in higher levels in mouse lungs.

Interestingly, in rat lungs, isoprostane compounds, typically released under oxidative stress, were either not detectable or detected at low concentrations, whereas mouse lungs manifested significantly higher concentrations (Table 3).

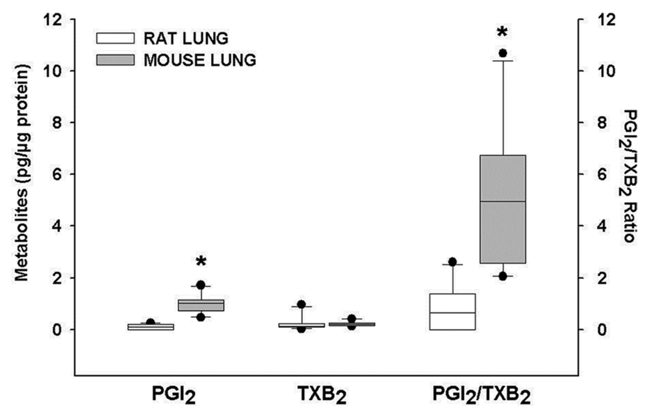

Figure 2: Concentrations of prostacyclin and thromboxane metabolites and their ratio in rat and mouse lungs. Mouse lungs had higher concentrations of PGI_2 metabolites than rat lungs. Similarly, PGI_2/TXB_2 ratio was higher in mouse lungs. PGI_2: Prostacyclin; TXB_2: Thromboxane B_2. *$P < 0.001$.

DISCUSSION

We established that basal levels of select bioactive mediators derived from arachidonic acid are generated in the lungs of rats and mice and confirmed our hypothesis that there are interspecies pulmonary eicosanoid concentration differences that associate with interspecies differences in the severity of pulmonary hypertension.

5-, 8-, 12-, and 15-HETE were present in all samples and their levels were significantly higher in mouse lung tissue. HETEs can be synthesized by LOXs and cytochrome P450 isoenzymes. 5-HETE is an enzymatic product of 5-LOX, which can participate in the pathogenesis of experimental pulmonary hypertension. 5-LOX overexpression in rat lungs worsens the severity of pulmonary hypertension in the monocrotaline model and administration of 5-LOX inhibitors prevents the development of the disease.[18] In addition, 5-LOX inhibitors attenuate the growth of human pulmonary artery endothelial cells in culture, suggesting that this enzyme may modulate the response of the lung vasculature to experimental pulmonary hypertension.

12-HETE and 15-HETE are products of 12/15-LOX. 12-HETE is the major product of 12/15-LOX in rodents and is also generated by a dedicated 12-LOX. 12-HETE stimulates proliferation of pulmonary artery smooth muscle cells through a MAPK-dependent mechanism, possibly having a role in the remodeling process in experimental pulmonary hypertension. We previously reported an increase in 12-LOX gene expression and an upregulation of 12-LO protein in the lungs of hypoxic rats.[4] 15-HETE is a precursor for lipoxins, a family of proresolvin mediators[19]

and has anti-inflammatory effects. 15-HETE effects on the vasculature, on the contrary, are conflicting. In some model systems, 15-HETE can mediate hypoxic pulmonary vasoconstriction[20,21] and displays antiapoptotic effects on pulmonary artery smooth muscle cells.[22] In other model systems, 15-HETE demonstrates vasodilator properties on both systemic and pulmonary beds.[23,24] Lastly, 8-HETE was also detected in both rat and mouse lungs, although its biologic properties on the lung vasculature are less extensively characterized.

Chronic hypoxia in mice is associated with only minimal vascular remodeling, certainly less than in rats.[12] The degree of remodeling parallels the degree of lung inflammation, with minimal inflammatory reaction in mice compared with rats. Similarly, monocrotaline causes severe pulmonary hypertension characterized by severe inflammation and remodeling in rats, but not in mice.[12] We found that two HETEs with vasoconstrictor and/or proliferative actions on the pulmonary vasculature, 5-and 12-HETE, are in higher concentrations in mouse than in rat lungs. While our results do not support the notion that basal HETE levels influence the response to pulmonary vascular stimuli, the significance of higher levels of 5-and 12-HETE as well as 15-HETE and 8-HETE in mouse lung under basal conditions is not known.

Human pulmonary hypertension is associated with an imbalance of urinary PGI_2 and TXA_2 metabolites favoring TXA_2.[9] Moreover, expression of PGI_2 synthase is decreased in the lungs of patients with pulmonary hypertension.[25] In addition, mice overexpressing PGI_2 synthase are protected against the development of hypoxic pulmonary hypertension.[26] Therefore, it was of interest to determine lung concentrations of PGI_2 and TXA_2, as well as their ratio. TXA_2 is unstable in vivo and is hydrolyzed within 30 seconds to TXB_2. Similarly, PGI_2 converts rapidly into several metabolites in vivo. We were able to measure TXB_2 and two metabolites of PGI_2, 6-keto-$PGF_{1\alpha}$ and 2,3-dinor-6-keto-$PGF_{1\alpha}$. We determined that the total PGI_2 metabolites and the ratio of the PGI_2 metabolites and TXB_2 were significantly higher in mouse lungs, and this increased ratio was mostly due to higher concentrations of PGI_2 metabolites in mouse tissue. Of note, PGI_2 is generated by the endothelium of pulmonary arteries, while TXA_2 is mainly produced by activated platelets. The relative high concentration of PGI_2 in the mouse suggests an increased cyclooxygenase-2 expression, or activity, or a decreased PGI_2 metabolism in pulmonary endothelial cells. Since prior reports suggest an active role of PGI_2 and TXA_2 in the pathogenesis of pulmonary hypertension, it is possible that the mild degree of experimental pulmonary hypertension in mice is partly due to a high basal ratio of PGI_2/TXB_2.

Lastly, we determined basal levels of two products of the isoprostane pathway: 8-iso $PGF_{2\alpha}$ and 8-iso PGE_2. Isoprostane formation from arachidonyl moieties in phospholipids occurs in the setting of oxidative states and can disrupt biological membranes.[27] Under basal conditions, isoprostanes are rapidly cleaved, released, metabolized and excreted. 8-iso $PGF_{2\alpha}$ is among the most abundant isoprostanes formed in vivo and is widely recognized as a biomarker of oxidative stress.[28] Our data suggest that mouse lungs had significantly higher basal levels of both 8-iso $PGF_{2\alpha}$ and 8-iso PGE_2 when compared with rat lungs, suggesting the existence of oxidative activity in mouse lungs under basal conditions. A third isoprostane, 8-iso-13,14-dihydro-15-keto-$PGF_{2\alpha}$, was not detected in any of the samples analyzed.

Limitations of our study include our inability to identify the cellular source of each eicosanoid in the lung, but reports from us and others suggest that the pulmonary vasculature is an active site of eicosanoid production. We did not determine which of the three pathways is more significant in the production of various HETEs in lung tissue: LOXs, cytochrome P450 isoenzymes, or cyclooxygenases. In addition, the column we selected did not permit chiral selective measurement of (R) and (S) enantiomers. More importantly, the present data cannot be directly extrapolated to susceptibility of these animals to the development of experimental PH. Further studies, such as determination of eicosanoid profiles with hypoxia-induced PH, are needed to clarify which of the eicosanoids described here modulate the vascular tone and cellular changes associated with PH.

In conclusion, we report for the first time, using a systematic screening approach through lipidomic analysis, a comparison of basal levels of select eicosanoids in rat and mouse lung. We show that arachidonic acid metabolism is active in rodent lung tissue and that there are significant interspecies differences in levels of select eicosanoids. These eicosanoids may participate in the maintenance and modulation of pulmonary vascular tone under basal conditions and during experimental vascular diseases. Lastly, we show that isoprostanes are present in mouse lung under basal conditions. Our determination of basal levels of select metabolites of arachidonic acid establishes the conditions with which to test whether differences in the levels of eicosanoids between species contribute to the differential responses to experimental pulmonary hypertension.

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