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

Kristen D. Sagliani1, Gregory G. Dolnikowski2, Nicholas S. Hill1, Barry L. Fanburg1, Bruce D. Levy3, and Ioana R. Preston1

1Pulmonary, Critical Care and Sleep Division, Tufts University School of Medicine, Tufts Medical Center, 2Mass Spectrometry Laboratory, Jean Mayer USDA HNRCA at Tufts University, and 3Pulmonary and Critical Care Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts, USA

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, PGE2, and PGI2, as well as isoprostane-E2 and -F2α. In addition, the PGI2/TXB2 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 PGI2/TXB2 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 (PGI2), a potent vasodilator which inhibits platelet aggregation and has antiproliferative and anti-inflammatory properties.[5] Conversely, thromboxane A2 (TXA2), produced from arachidonic acid via the action of thromboxane synthase, opposes the actions of PGI2 by promoting vasoconstriction, platelet aggregation, and smooth muscle cell proliferation.[6]

Decreased local production and availability of PGI2 have been implicated in the pathogenesis of pulmonary hypertension. Using a rat model, Rabinovitch et al.[7] showed that induction of PGI2 release prevents hypoxic pulmonary hypertension and vascular remodeling. In addition, translational research showed that patients with
pulmonary arterial hypertension (PAH) have an abnormally low ratio of urinary PGI₂ to TXA₂ 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 PGI₂[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. 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 (PGF₂α); 9S,11S-trihydroxy-5Z,13E-prostadienoic acid-cyclo[8S,12R] (8-iso-PGF₂α); 9S,11S,15S-trihydroxy-5Z,13E-prostadienoic acid-cyclo[8S,12R] (8-iso-PGF₂α)-d₄; 9S,11S,15S-trihydroxy-13E-prostaglandin D₂ (PGD₂); 9S,11S,15S-trihydroxy-13E-prostaglandin D₂-d₄ (PGD₂-d₄); 9S,11S-trihydroxy-13E-prostaglandin E₂ (PGE₂); 9S,11S-trihydroxy-13E-prostaglandin E₂-d₄ (PGE₂-d₄); 9S,11S-trihydroxy-thromboxa-5Z,13E-dien-1-oxic acid (TXB₂); 9-oxo-11R,15S-dihydroxy-5Z,13E-prostadienoic acid-cyclo[8S,12R] (8-iso-PGE₂α); 9S,11S,15S-trihydroxy-5Z,13E-prostadienoic acid (PGD₂); 9S,11S-trihydroxy-5Z,13E-prostadienoic acid (PGE₂); 9S,11S-trihydroxy-13E-prostadienoic acid (PGE₂); 9S,11S,15S-trihydroxy-13E-prostadienoic acid (PGD₂); 9S,11S,15S-trihydroxy-13E-prostadienoic acid (PGE₂). 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 20 µL aliquot of internal standard master mix (5-, 12-, 15-, HETE-d₄, PGE₂-d₄, TXB₂-d₄) 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.
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 x 2.00 mm x 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 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.

**Temperature** and ion-spray voltage were set at 350°C and -4500 V, respectively. Collision energy was determined for each specific compound. Using a valve 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\(_4\) internal standard. **HETEs**: hydroxyeicosatetraenoic acid; **MRM**: multiple reaction monitoring; **CE**: collision energy; CXP: collision exit potential; **DP**: declustering potential; \(t\): 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\(_2\) | 351.4 → 174.9 | -27.77 |
| 13,14-dihydro-15-keto-PGE\(_2\) | 351.4 → 233.2 | -14.0  |
| PGD\(_2\)   | 351.4 → 175.0 | -29.2  |
| 11β-PGF\(_2\) | 353.4 → 209.3 | -24.42 |
| 8-iso-PGF\(_2\) | 353.4 → 209.3 | -24.42 |
| PGG\(_2\)   | 353.4 → 193.0 | -32.0  |
| 8-iso-13,14-dihydro-15-keto-PGF\(_2\) | 353.4 → 183.0 | -33.71 |
| 6-keto-PGF\(_2\) | 369.4 → 245.1 | -35.71 |
| 2,3-dinor-6-keto-PGF\(_2\) | 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\(_2\), PGF\(_2\), and 11β-PGF\(_2\) 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).

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: PGE₂ (with one isomer and two metabolites); PGD₂ (with one metabolite); PGF₂α (with one isomer); two PGI₂ metabolites, one thromboxane metabolite (TXB₂), 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, PGI₂ metabolites were significantly higher in mouse than in rat lungs and it was found in very low concentrations. Mouse lungs had detectable levels of both isoprostane-E₂ and -F₂α; for abbreviations see Table 2.

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

| Internal Standard | Mouse Lung (N=10) | Rat Lung (N=10) |
|-------------------|-------------------|-----------------|
| 5-HETE-d₄         | 71±9              | 62 ± 22         |
| 12-HETE-d₄        | 71±10             | 80 ± 42         |
| 15-HETE-d₄        | 73±23             | 66 ± 15         |
| PGE₂-d₄           | 92±11             | 84 ± 37         |
| TXB₂-d₄           | 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) | 0.025 (0.022, 0.054) | 0.026 |
| 8-HETE         | 0.039 (0.02, 0.053)  | 0.048 (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.

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

| PGs | Rat (N=10) | Mouse (N=10) | P value |
|-----|------------|--------------|---------|
| PGE₂       | 0.03 (0.02, 0.03) | 0.4 (0.3, 0.5) | 0.003 |
| 11β-PGE₂   | 0.07 (0.00, 0.1)  | 0.3 (0.2, 0.3)  | 0.014 |
| 15-keto-PGE₂ | ND             | 0.029 (0.02, 0.06) | <0.001 |
| 13,14-dihydro-15-keto-PGE₂ | ND | 0.08 (0.065, 0.097) | <0.001 |
| PGD₂       | ND           | 0.09 (0.03,0.2) | <0.001 |
| 13,14-dihydro-15-keto-PGD₂ | ND | 0.095 (0.07, 0.1) | <0.001 |
| PGF₂α      | ND           | 0.09 (0.08, 0.12) | <0.001 |
| 11β-PGF₂α  | ND           | 0.046 (0.043, 0.07) | <0.001 |
| 6-keto-PGF₂α | 0.036 (0.00, 0.206) | 0.72 (0.67, 0.93) | <0.001 |
| 2,3-dinor-6-keto-PGF₂α | ND | 0.214 (0.132, 0.27) | <0.001 |
| Sum of PGI₂ metabolites | 0.036 (0.00, 0.206) | 1.002 (0.35, 0.11) | <0.001 |
| TXB₂       | 0.13 (0.087, 0.20) | 0.20 (0.16, 0.24) | NS      |
| PGI₂/TXB₂  | 0.327 (0.00, 0.129) | 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 TXB₂ had similar levels in both species. PG: prostaglandin; PGI₂: prostacyclin; TXB₂: thromboxane B₂.

**Table 3: Concentrations of select isoprostanes in rodent lung**

| Isoprostanes | Rat (N=10) | Mouse (N=10) | P value |
|--------------|------------|--------------|---------|
| 8-iso-PGE₂  | 0.0003 (0.0002, 0.0007) | 0.004 (0.003, 0.004) | 0.003 |
| 8-iso-PGF₂α | ND         | 0.02 (0.017, 0.023) | <0.001 |
| 8-iso-13,14-dihydro-15-keto-PGF₂α | ND | ND | NS |

Concentrations of select isoprostanes (pg/μg protein) are reported as median (25-75% confidence interval). Isoprostane-E₂ was the only compound detected in rat lungs and it was found in very low concentrations. Mouse lungs had detectable levels of both isoprostane-E₂ and -F₂α. For abbreviations see Table 2.
lungs and TXB₂, the TXA₂ metabolite had similar levels. Accordingly, there was a significantly higher PGI₂/TXB₂ ratio in mouse than in rat lungs (Fig. 2). Lastly, PGE₂, a bronchodilator and vasodilator, 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).

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. 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. 15-HETE is a precursor for lipoxins, a family of proresolvin mediators.

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.

Figure 2: Concentrations of prostacyclin and thromboxane metabolites and their ratio in rat and mouse lungs. Mouse lungs had higher concentrations of PGI₂ metabolites than rat lungs. Similarly, PGI₂/TXB₂ ratio was higher in mouse lungs. PGI₂: Prostacyclin; TXB₂: Thromboxane B₂. *P < 0.001.
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 antiproliferative 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 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$_2$.[8] 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$_{1α}$ and 2,3-dinor-6-keto-PGF$_{1α}$. 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$_{2α}$ and 8-iso PGE$_{2α}$. 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$_{2α}$ 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$_{2α}$ and 8-iso PGE$_{2α}$ 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$_{2α}$, 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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