Published in final edited form as:
Pediatr Res. 2009 January ; 65(1): 33–38. doi:10.1203/PDR.0b013e31818a1d0a.

Differential Responses in the Lungs of Newborn Mouse Pups Exposed to 85% or >95% Oxygen

Lynette K. Rogers, **Trent E. Tipple**, **Leif D. Nelin**, and **Stephen E. Welty**
Department of Pediatrics, Ohio State University, Columbus Ohio 43205 USA

Abstract

Premature infants often develop serious clinical complications associated with respiratory failure and hyperoxic lung injury that include lung inflammation, and alterations in lung development. The goal of these studies is to test the hypothesis that there are differences in the course of lung injury in newborn mice exposed to 85% or >95% oxygen that provide models to address the differential effects of oxidation and inflammation. Our results indicate differences between the 85% and >95% O2 exposure groups by day 14 in weight gain and lung alveolarization. Inflammation, assessed by neutrophil counts, was observed in both hyperoxia groups by day 3 but was dramatically greater in the >95% O2 exposed groups by day 14 and associated with greater developmental deficits. Cytoplasmic phospholipase A_2, cyclooxygenase-2, and 5-lipoxygenase levels were elevated but no patterns of differences were observed between exposure groups. Prostaglandins (PG) D_2, E_2, and $F_{2\alpha}$ were increased in the tissues from mouse pups exposed to >95% O2 at 7 days indicating a differential expression of COX-2 products. Our data indicate that there are differences in the models of 85% or >95% O2 exposure and these differences may provide mechanistic insights into hyperoxic lung injury in an immature system.

Keywords

Hyperoxia; newborn; eicosanoids; inflammation

The development of bronchopulmonary dysplasia (BPD), a form of chronic lung disease associated with prematurity, occurs in immature lungs upon exposure to supplemental oxygen, mechanical ventilation, and inflammatory cell infiltration. The structural and biochemical changes that occur during exposure to hyperoxia are well described in animal models (1–6).

Mice pups are born in the saccular stage of lung development and decreased alveolarization is a prevalent characteristic in mouse pups exposed to hyperoxia during the early post-natal period. Previous studies using 85% and >95% O2 exposures have reported disruption of key growth events and aberrant alveolarization that may lead to permanent deficits in lung growth (7–9) but comparative studies have not been reported. Neonatal mice often succumb to >95% O2 with robust inflammatory responses while models using less than 90% O2 have demonstrated less inflammation and limited lethality (7). It is possible that the different

Corresponding Author: Lynette K. Rogers, Ph.D., Center for Perinatal Research, The Research Institute at Nationwide Children's Hospital, 700 Children's Drive, Columbus, Ohio 43205, Department of Pediatrics, Ohio State University, Tel: (614) 355-3456, Fax: (614) 355-3455, Email: rogersl@ccri.net.

outcomes in these types of studies are related to more exaggerated lung inflammation in newborn mice exposed to >95% oxygen than in newborn mice exposed to <90% oxygen, and our hypothesis is that the differences in lung inflammatory responses between these two models are related to differences in the formation of lipid inflammatory mediators.

The lung has large quantities of cellular membrane glycerophospholipids which contain esterified arachidonic acid and provide a source for the formation of biologically active arachidonic acid metabolites or eicosanoids (10). Phospholipases (PL) are responsible for cleavage of esterified fatty acids from membrane phospholipids. Cyclooxygenases (COX) form prostaglandins (PG) and thromboxanes (TBX), which have potent inflammatory and vasoconstrictive properties. Lipoxygenases (LO) produce hydroxyeicosatetraenoic acids (HETE) that may function as precursors for other biologically active lipid products or as ligands for receptors. 5-LO is specifically responsible for leukotriene (LT) formation and often associated with neutrophil chemotaxis (Figure 1).

The goal of these studies is to test the hypothesis that differences in the course of injury in newborn mice exposed to 85% and >95% oxygen exist and provide distinctly different models to address developmental deficits associated with hyperoxic lung injury (85%O2) separate from the additive effects of inflammation and developmental deficits associated with >95% O2. Furthermore, these studies test the hypothesis that the differences observed between 85% and >95% O2 exposure are in part due to activation of pro-inflammatory lipid mediators. Careful temporal analyses of hyperoxia exposure in newborn pups may provide clues that lead to the design of new interventions for treatment in premature infants.

Methods

Animal Models

Animal study protocols were approved by the IACUC at Columbus Children's Research Institute. Pups were randomized and equally distributed between 2 pregnant C3H/HeN dams delivering within 12 h. One dam and litter was placed in a plexiglass chamber containing a 10 L/min flow of 85% or >95% (daily average 98%) O2 while the corresponding dam and litter were placed in room air (RA). Twenty-four hours of hyperoxia exposure was designated as day 1. On 1, 3, 7, or 14 days of life, the pups were euthanized by intraperitoneal injections of 200 mg/kg of sodium pentobarbital and the tissues harvested.

Morphometric and Digital Image Analysis

Morphometric analyses were performed as described by Park et al. (8). The following parameters were determined: number of complete terminal airways per field of view; average terminal airway area; and average terminal airway perimeter using Image J, version 1.37 software (National Institutes of Health, http://rsb.info.gov/ij). Manual measurements are made of the numbers of secondary crests per image and the septal thicknesses. Septal thickness was assessed by linear measurements of the septum at a 90°angle, using at least five random measurements per field, and five fields per slide (animal).

Immunohistochemistry for Neutrophil Quantification

Neutrophil counts were performed on paraffin embedded, anti-neutrophil stained mouse tissues. The primary antibody was rat anti-mouse neutrophils (Serotec, Kingston, UK) at a dilution of 1:2500 and secondary antibody was rabbit anti-rat, mouse adsorbed (Vector, catalog #BA-4001) at a dilution of 1:200. The slides were then counterstained with Richard Allen hematoxylin.

Western blots

Frozen lung tissues were homogenized and proteins (25–50 μg) were separated by 12% SDS-PAGE (Invitrogen, Carlsbad, CA) and transferred to polyvinylidene difluoride membranes. Membranes were probed with antibodies to cytoplasmic PLA2 (rabbit anti-mouse, 1:1000, Cell Signaling, Danvers MA), COX-2 (rabbit anti-mouse, 1:200, Abcam, Cambridge MA), or 5-LO (mouse monoclonal, 1:1000, BD Bioscience, San Jose, CA) and the appropriate secondary antibody, anti-rabbit (1:10000, BioRad, Hercules CA) or anti-mouse (1:15000, BioRad, Hercules CA). Blots were assessed with ECL detection and expression levels were quantitated by densitometry using Image Quant software, version 5.0 (Molecular Dynamics). The density of the band for the protein of interest was normalized to the density of β-actin protein (mouse monoclonal 1:5000, Abcam; goat anti-mouse, 1:15000, BioRad).

LC/MS/MS Lipid Analysis

Lung tissues were homogenized and each sample was spiked with an internal standard solution (deuterated standards from each lipid group) then extracted using the Bligh-Dyer technique. Eicosanoids were analyzed by LC/MS/MS on an Applied Biosystems 4000 QTrap equipped with a Shimadzu HPLC. Separation was achieved using a Zorbax SB-C18 column, a flow rate of 0.3 mL/min, and a gradient of 8.3 mM acetic acid, pH 5.7 (mobile phase A) and acetonitrile: 2-propanol (50:50) (mobile phase B) as follows: 3 min hold at 15%B, 10 min linear to 55%B, 15 min linear to 80%B. The samples are analyzed in negative ionization mode using Multiple Reaction Monitoring (MRM). Individual calibration curves are generated for each group of analytes, and sample concentrations are calculated using isotope dilution corrections.

Statistics

Data collected from analyses was analyzed by two-way ANOVA with time and exposure as independent variables. Individual differences were detected using modified t-tests post-hoc with $p<0.05$ as significant. All analyses were performed with SPSS Windows version 15.0 (Chicago, IL).

Results

Mouse pups exposed to 85% or >95% O2 grew more slowly (Figure 2) and exhibited less lung alveolarization (Figures 3A and B) than did their respective room air (RA) exposed littermates. By day 7, pups in both hyperoxia exposed groups weighed less than the pups in the RA control group and but by day 14 the weights of the mice in the 85% O2 exposed group were greater than the >95% O2 exposed group but still less than the RA controls. Those pups exposed to 85% O2 exhibited a lag in growth in the 1 to 7 day period but grew at the same rate as the RA exposed animals during the 7 to 14 day period. Those pups exposed to >95% O2 did not demonstrate an early growth delay (days 1–3) but a growth delay was obvious at 7 and 14 days. No mortality was observed at 14 days.

Histological analyses of lung tissue sections obtained from pups exposed to 85%, >95% and RA indicated decreased alveolarization at 7 and 14 days in pups exposed to hyperoxia (Figure 3A). Morphometric measurements of the histological sections demonstrated specific deficits in lung growth in the pups exposed to hyperoxia (Figure 3B). In the RA controls, the number of terminal airspaces or alveoli (Figure 3B1) and secondary crests (Figure 3B2) increased, while terminal airspace area (Figure 3B3), perimeter (Figure 3B4), and septal thickness (Figure 3B5) decreased through the first 14 days of life. Both hyperoxia exposed groups had larger terminal airspace area and fewer secondary crests by 3 days of exposure and lower terminal airspace number and larger perimeters by 7 days than the corresponding RA group. Differences in analyzed morphometric perimeters of pup tissues exposed to 85% and >95% O2 became evident between 7 and 14 days with the 85% exposure group demonstrating more secondary

crests and less alveolar area and smaller perimeters by 14 days. Septal thickness was greater in the 85% O2 exposed pups at all times than in the room air controls. Septal thickness decreased in the RA animals from day 7 to day 14. Pups exposed to >95% O2 had septal thickness similar to room air controls until day 14 when septal thickness was significantly greater in >95% O2 exposed group than in RA group.

As an assessment of inflammation, lung sections were stained with an anti-neutrophil antibody and number of neutrophils per high power field was counted. At day 3 and 7, there were more neutrophils in the lungs of the pups exposed to 85% or >95% O2 than in the lungs of pups that remained in RA. By day 14 there were no differences in the number of neutrophils between the RA and the 85% O2 groups, but those pups exposed to >95% O2 had substantially higher numbers of neutrophils present in their lung tissues (Figure 4).

Cytoplasmic PLA_2 ($cPLA_2$) protein levels were not different among groups for days 1 and 3 but were greater in lung tissues after 7 days exposure to 85% or >95% O2 than in RA controls (Figure 5). Interestingly, the higher levels of $cPLA_2$ protein were maintained through 14 days in the >95% O2 exposed pup tissues but the levels of $cPLA_2$ in the 85% O2 exposed pup tissues were less than the >95% O2 exposed tissues and not different than room air at 14 days.

COX-2 levels were similar in all treatment groups at days 1 and 3 but COX-2 levels were greater in lung tissues of mouse pups exposed to 85% or >95% O2 for 7 or 14 days of exposure than in the corresponding RA controls (Figure 6). The hyperoxia-induced increases in COX-2 levels were equivalent in both 85% and 95% O2 exposed pup tissues.

Similar to COX-2, 5-LO levels were not different among groups at days 1 and 3. However, at day 7, the 5-LO levels of tissues from pups exposed to 85% O2 were similar to control levels but the levels in the lung tissues of >95% O2 exposed pups were greater than RA controls or the 85% O2 groups (Figure 7). By day 14, lung tissues from both O2 exposed groups indicated substantially greater 5-LO levels than the RA day 14 group.

Metabolites of the COX-2 pathways, PGD_2, PGE_2, $PGF_{2\alpha}$ and $TBXB_2$ were measured as evidence of COX-2 enzymatic activity (Table 1). At days 1 and 3 there was a trend toward higher levels of COX-2 metabolites in the 85% O2 exposed pup tissues than in RA or >95% O2 tissues, but this trend was not significant in most cases. However, a robust elevation was observed at 7 days in the tissues obtained from pups exposed to >95% O2 that was not observed in the 85% O2 exposed group. At 14 days, the PGD2 levels were elevated in both the 85% O2 and the >95% O2 groups. Interestingly, the elevated levels of PGE_2 and $PGF_{2\alpha}$ did not persist in the lungs of pup exposed to 85% or >95% O2 for 14 day of exposure. On the other hand, $TBXB_2$ demonstrated a modest developmental increase and a slight elevation above control levels at day 7 in the >95% O2 exposed group.

In spite of increases in 5-LO protein levels, no appreciable differences in 5-HETE levels were observed among treatment groups. LTB_4, LTC_4, LTD_4, and LTE_4 levels were measured in the pup lung tissues but were not consistently above the limits of detection for the methods described.

Discussion

The goal of these studies was to identify distinct differences in the course of injury in newborn mice expose to 85% or >95% O2. Detrimental effects of hyperoxic exposure were easily observable in newborn animals as deficits in overall body growth and these effects were different depending upon the oxygen concentration (Figure 2). Growth deficits have been reported in several animal models exposed to a variety of O2 concentrations (6,11,12). Although other studies have observed substantial mortality at earlier time points and lower O2

concentrations (7), no mortality was observed in these studies. These differences in mortality are likely attributable to the decreased susceptibility of the C3H/HeN mouse strain to hyperoxic injury. The apparent resumption of growth in the 85% O2 exposed pups after 7 days may indicate a time frame for the development of resolution or adaptive mechanisms.

Interestingly, both hyperoxia-exposed groups demonstrated evidence of arrested lung development as early as three days of exposure. This finding would support the theory that the initiating effects of hyperoxic exposure on lung growth occur very early in the time course before other physiological parameters are evident but coinciding with the first evidence of neutrophil infiltration. However, the differential responses between the hyperoxia-treated groups occur later during the second week of exposure, and suggest adaptive responses in the mice exposed to 85% O2 (Figure 3B) but greater inflammatory responses in the mice exposed to >95% oxygen (Figure 4). These models may be relevant to human premature infants in that pronounced oxidation is noted early in infants that develop BPD (13, 14), and the persistence of lung inflammation is associated with more severe disease (15).

The effects of hyperoxia on lung growth and development in our studies are consistent with those that have been previously described (Figure 3A) (7, 8, 16–18). Lung tissue morphologic comparisons between 85% and >95% O2 demonstrate similar patterns of delayed growth at day 1 and 3 but between 7 and 14 days of exposure lung tissues from the 85% exposure group resumed patterns of growth similar to the room air controls. Lung tissues from the 95% exposure group have even more pronounced defects in alveolar development and larger airspace areas that might represent tissue destruction rather than an isolated effect of deficits in lung development (Figure 3B). The apparent decrease in alveolar development we observed at 14 days of >95% O2 exposure could be explained as hyperoxia-induced cell death (12, 19), but further characterization of this deficit is beyond the scope of the present study. The greater septal thickness in the 85% O2 exposed pups than in the RA pups at all days may indicate inhibition of pathways associated with septal thinning. More important than the relative differences between exposures is the fact that the septa did not thin between days 1 and 14 in either hyperoxia groups but did show signs of thinning in the RA pups.

Increases in the numbers of neutrophils in the lung due to hyperoxic exposure are considered indices of inflammation (6,7,20,21). The substantially greater number of neutrophils at day 14 in the tissues from pups exposed to >95% O2 than in those exposed 85% O2 or room air (Figure 4) indicate that there is greater inflammation in the lungs of the pups exposed to >95% O2. These differences in inflammation may indicate induction of inflammatory resolution pathways in the 85% O2 exposed pups that fail to occur in the 95% O2 exposed pups.

Lipid mediators possess potent pro- and anti-inflammatory properties that could influence the responses to hyperoxia in the lung. $CPLA_2$ levels have been associated with deficiencies in clara cell secretory protein, mechanical ventilation, and LPS-induced lung injury (22,23). We observed substantial increases in $cPLA_2$ protein levels in both 85% and >95% O2 exposed groups at day 7 that persisted in the >95% O2 group through day 14 (Figure 5). Increases in $cPLA_2$ levels would be the first step in the propagation of metabolically active lipids and the decreased levels observed in the 85% O2 exposed group at 14 days further support the induction of adaptive pathways between 7 and 14 days of exposure.

COX-2 is produced in response to inflammatory stimuli but low levels of constitutive expression are evident in the lung (24). COX-2 and COX-2 metabolites have been shown to be involved in acute lung injury in animal models of BPD (25–27). In the current study, COX-2 levels in hyperoxia-exposed pups were elevated by day 7 in both oxygen exposed groups (Figure 6) and this elevation persisted through 14 day. A doubling of COX-2 levels by 7 days

in lung tissues from hyperoxia exposed pups suggests the possibility of increased prostaglandin and/or thromboxane synthesis in the lungs of these animals.

The greatest increases in the PGs measured in these studies were at 7 days of hyperoxia exposure. However, the increases in PG levels were observed in the tissues from animals exposed to >95% O2 only. This suggests differential regulation of the COX-2 pathway between 85% and >95% O2 exposure. Whether this finding represents the inhibition of other enzymes necessary for synthesis of PGs or alternative regulation of COX-2 is unknown and necessitates further investigations into the adaptive mechanisms associated with hyperoxia in these models.

LTs are increased in animals exposed to hyperoxia, and inhibition of 5-LO activities have prevented deficits in alveolarization (28). We observed no differences in 5-LO levels for the first 3 days of O2 exposure, but at day 7 increases were evident in the >95% O2 group only (Figure 7). The increased levels of 5-LO in the 85% O2 exposed group at 14 days, in the absence of increased numbers of neutrophils, are not easily explained but may indicate alternative activities of 5-LO or increases in inflammatory resolution pathways in the lungs of these animals.

LT levels were not consistently detectable in the tissues of the newborn mouse pups and would likely to be at the highest levels in either the BAL or blood. However, technical limitations make these studies difficult because of issues in obtaining sufficient quantities of BAL or blood from these extremely small pups. We did measure 5-HETE levels as an indicator of 5-LO activity and found no differences between exposures throughout the time course of hyperoxia. These findings would suggest that the 5-LO levels measured in the tissues are likely to be coming from neutrophils that are primarily present in the air spaces and exerting their effects on the cells and proteins at or near the gas exchange surface. Consequently, the relative abundance of the products of 5-LO may be difficult to measure in the background of a whole lung homogenate.

The mechanisms associated with the adaptive capabilities of mouse pups exposed to sub-lethal concentrations of oxygen are not easily understood. Our data indicate that pups exposed to 85% O2 are able to resume normal body growth rates after 7 days, but do not recover to the levels of their RA counterparts by 14 days. Neutrophil accumulations in the lungs of pups exposed to 85% O2 are remarkably less than in the lungs of pups exposed to >95% O2 by day 14. Our original hypothesis that lipid metabolizing enzymes would be greater in the pups exposed to >95% O2 than those exposed to 85% O2 or RA was refuted as induction of $cPLA_2$, COX-2 and 5-LO was for the most part similar in both hyperoxia-exposed groups. However, measurable increases in prostaglandin levels were primarily evident in the 95% O2 exposed pups at 7 days suggesting alternative regulation of COX-2 metabolites and suppression of downstream enzymes in the 85% O2 exposed pups. Collectively, these data indicate that key events take place in the lungs of mouse pups at between 7 and 14 days of exposure to 85% O2 that result in either adaptation or inflammatory resolution. Alternatively, the differences in responses to hyperoxia between the two exposure groups could represent a continuum of injury mediated by differential contributions of oxidative and inflammatory mechanisms between the exposure groups.

Our data clearly indicate a difference in response to 85% and >95% O2 exposure however, further studies are needed to define the mechanisms responsible for these differences. Understanding the mechanisms associated with these events could lead to therapeutic interventions against hyperoxic lung injury in premature infants and the subsequent development of BPD.

Acknowledgements

The authors would like to acknowledge the technical support provided by Xiaomei Meng, Molly Augustine, and Kathryn Heyob.

Financial Support: Partially Funded by NHLBI, HL068948

Abbreviations

BPD
bronchopulmonary dysplasia

COX-2
cyclooxygenase-2

cPLA2
cytoplasmic phospholipase A2

LO
lipoxygenase

LT
leukotriene

PG
prostaglandin

RA
room air

TBX
thromboxane

References

1. Wispe JR, Roberts RJ. Molecular basis of pulmonary oxygen toxicity. Clin Perinatol 1987;14:651–666. [PubMed: 3311543]
2. O'Donovan DJ, Rogers LK, Kelley DK, Welty SE, Ramsay PL, Smith CV. CoASH and CoASSG levels in lungs of hyperoxic rats as potential biomarkers of intramitochondrial oxidant stresses. Pediatr Res 2002;51:346–353. [PubMed: 11861941]
3. Tipple TE, Welty SE, Rogers LK, Hansen TN, Choi YE, Kehrer JP, Smith CV. Thioredoxin-related mechanisms in hyperoxic lung injury in mice. Am J Respir Cell Mol Biol 2007;37:405–413. [PubMed: 17575077]
4. Wong YL, Smith CV, McMicken HW, Rogers LK, Welty SE. Mitochondrial thiol status in the liver is altered by exposure to hyperoxia. Toxicol Lett 2001;123:179–193. [PubMed: 11641046]
5. Zhao B, Ramsay PL, Park MS, Welty SE, De MA. In vivo and in vitro analysis of hyperoxia-induced gene expression in mouse lung and mouse transformed Clara cells. Ann N Y Acad Sci 2000;923:346–347. [PubMed: 11193776]
6. Crapo JD, Barry BE, Foscue H, Shelburne J. Structural and Biochemical Changes in Rat Lungs Ocurring During Exposures to Lethal and Adaptive Doses of Oxygen. Am Rev Respir Dis 1980;122:123–143. [PubMed: 7406333]
7. Warner BB, Stuart LA, Papes RA, Wispe JR. Functional and pathological effects of prolonged hyperoxia in neonatal mice. Am J Physiol 1998;275:L110–L117. [PubMed: 9688942]
8. Park MS, Rieger-Fackeldey E, Schanbacher BL, Cook AC, Bauer JA, Rogers LK, Hansen TN, Welty SE, Smith CV. Altered expressions of fibroblast growth factor receptors and alveolarization in neonatal mice exposed to 85% oxygen. Pediatr Res 2007;62:652–657. [PubMed: 17957151]

9. Rehan VK, Wang Y, Patel S, Santos J, Torday JS. Rosiglitazone, a Peroxisome Proliferator-Activated Receptor-g Agonist, Prevents Hyperoxiz-Induced Neonatoal Rat Lung Injury In Vivo. Pediatr Pulmonol 2006;41:558–569. [PubMed: 16617452]
10. Nakamura T, Henson PM, Murphy RC. Occurrence of oxidized metabolites of arachidonic acid esterified to phospholipids in murine lung tissue. Anal Biochem 1998;262:23–32. [PubMed: 9735144]
11. Pappas CT, Obara H, Bensch KG, Northway WH Jr. Effect of prolonged exposure to 80% oxygen on the lung of the newborn mouse. Lab Invest 1983;48:735–748. [PubMed: 6855196]
12. McGrath-Morrow SA, Stahl J. Apoptosis in neonatal murine lung exposed to hyperoxia. Am J Respir Cell Mol Biol 2001;25:150–155. [PubMed: 11509323]
13. Ogihara T, Hirano K, Morinobu T, Kim HS, Hiroi M, Ogihara H, Tamai H. Raised concentrations of aldehyde lipid peroxidation products in premature infants with chronic lung disease. Arch Dis Child Fetal Neonatal Ed 1999;80:F21–F25. [PubMed: 10325806]
14. Nycyk JA, Drury JA, Cooke RW. Breath pentane as a marker for lipid peroxidation and adverse outcome in preterm infants. Arch Dis Child Fetal Neonatal Ed 1998;79:F67–F69. [PubMed: 9797630]
15. Speer CP. Inflammation and bronchopulmonary dysplasia. Semin Neonatol 2003;8:29–38. [PubMed: 12667828]
16. Massaro D, Massaro GD. Invited Review: pulmonary alveoli: formation, the “call for oxygen,” and other regulators. Am J Physiol Lung Cell Mol Physiol 2002;282:L345–L358. [PubMed: 11839527]
17. Massaro GD, Olivier J, Dzikowski C, Massaro D. Postnatal development of lung alveoli: suppression by 13% O2 and a critical period. Am J Physiol 1990;258:L321–L327. [PubMed: 2360645]
18. Alejandre-Alcazar MA, Kwapiszewska G, Reiss I, Amarie OV, Marsh LM, Sevilla-Perez J, Wygrecka M, Eul B, Kobrich S, Hesse M, Schermuly RT, Seeger W, Eickelberg O, Morty RE. Hyperoxia modulates TGF-beta/BMP signaling in a mouse model of bronchopulmonary dysplasia. Am J Physiol Lung Cell Mol Physiol 2007;292:L537–L549. [PubMed: 17071723]
19. Dieperink HI, Blackwell TS, Prince LS. Hyperoxia and apoptosis in developing mouse lung mesenchyme. Pediatr Res 2006;59:185–190. [PubMed: 16439576]
20. Ramsay PL, Smith CV, Geske RS, Montgomery CA, Welty SE. Dexamethasone enhancement of hyperoxic lung inflammation in rats independent of adhesion molecule expression. Biochem Pharmacol 1998;56:259–268. [PubMed: 9698081]
21. Crapo JD. Morphologic changes in pulmonary oxygen toxicity. Annu Rev Physiol 1986;48:721–731. [PubMed: 3518622]
22. Yoshikawa S, Miyahara T, Reynolds SD, Stripp BR, Anghelescu M, Eyal FG, Parker JC. Clara cell secretory protein and phospholipase A2 activity modulate acute ventilator-induced lung injury in mice. J Appl Physiol 2005;98:1264–1271. [PubMed: 15608088]
23. Nagase T, Uozumi N, Aoki-Nagase T, Terawaki K, Ishii S, Tomita T, Yamamoto H, Hashizume K, Ouchi Y, Shimizu T. A potent inhibitor of cytosolic phospholipase A2, arachidonyl trifluoromethyl ketone, attenuates LPS-induced lung injury in mice. Am J Physiol Lung Cell Mol Physiol 2003;284:L720–L726. [PubMed: 12505870]
24. Simmons DL, Botting RM, Hla T. Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. Pharmacol Rev 2004;56:387–437. [PubMed: 15317910]
25. Fukunaga K, Kohli P, Bonnans C, Fredenburgh LE, Levy BD. Cyclooxygenase 2 plays a pivotal role in the resolution of acute lung injury. J Immunol 2005;174:5033–5039. [PubMed: 15814734]
26. Hodges RJ, Jenkins RG, Wheeler-Jones CP, Copeman DM, Bottoms SE, Bellingan GJ, Nanthakumar CB, Laurent GJ, Hart SL, Foster ML, McAnulty RJ. Severity of lung injury in cyclooxygenase-2-deficient mice is dependent on reduced prostaglandin E(2) production. Am J Pathol 2004;165:1663–1676. [PubMed: 15509536]
27. Park GY, Christman JW. Involvement of cyclooxygenase-2 and prostaglandins in the molecular pathogenesis of inflammatory lung diseases. Am J Physiol Lung Cell Mol Physiol 2006;290:L797–L805. [PubMed: 16603593]
28. Manji JS, O’Kelly CJ, Leung WI, Olson DM. Timing of hyperoxic exposure during alveolarization influences damage mediated by leukotrienes. Am J Physiol Lung Cell Mol Physiol 2001;281:L799–L806. [PubMed: 11557583]

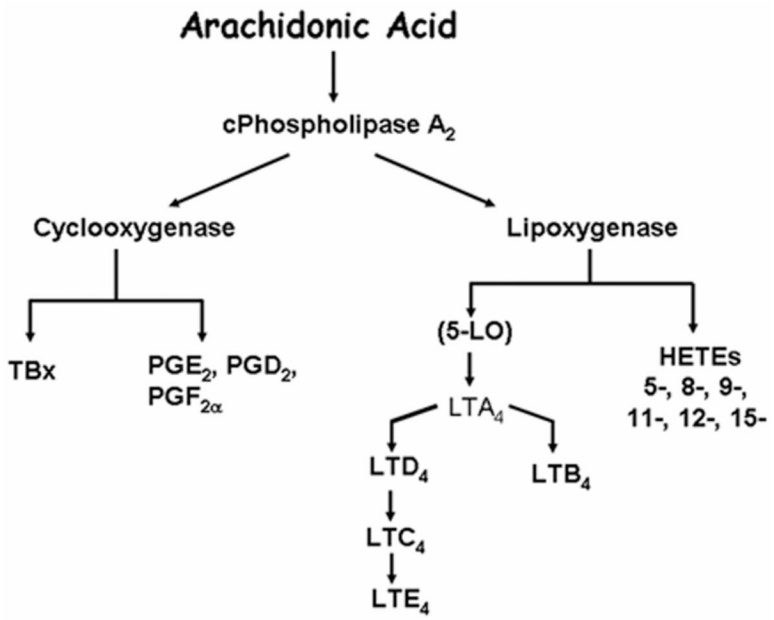

Figure 1.
Simplified scheme of arachidonic acid metabolism.

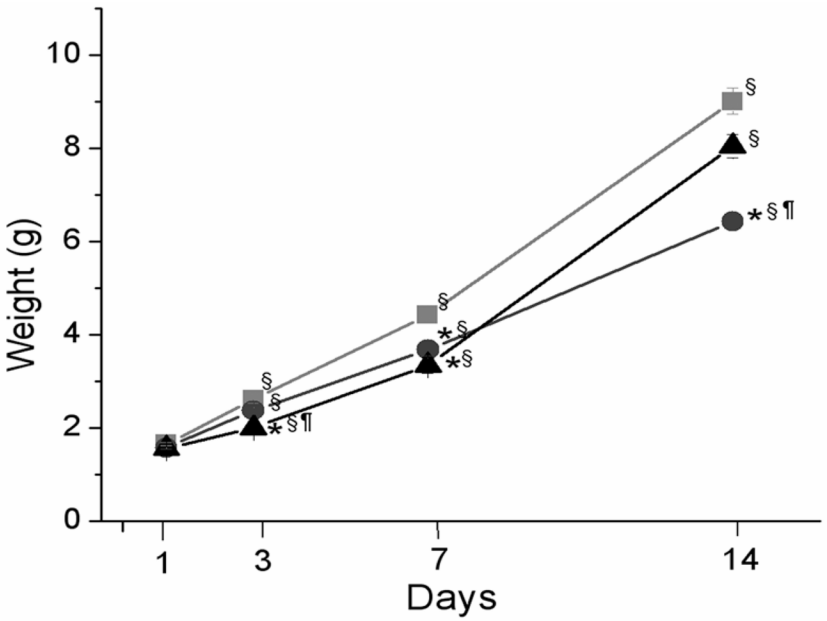

Figure 2.

Effects of 85% (▲) or >95% O2 (●) or room air (■) on body weight in newborn mice. Pups were treated as described in Methods. Results are reported as mean ± SD. Data were assessed by two-way ANOVA, with modified t-test post hoc. An effect of day, exposure, and an interaction was noted, $p<0.05$, n=5–32. *different between RA and O2 exposed animals on the same day, §different from day 1 same treatment, ¶different between 85% and >95% O2 same day.

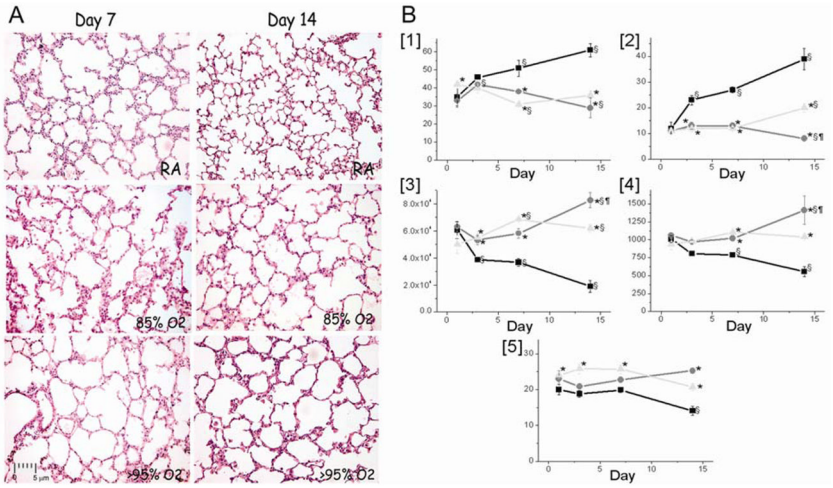

Figure 3.

A) Histology of mouse pup lung tissues exposed to 85% (▲) or >95% O2 (●), or RA (■). Pups were treated as described in Methods and fixed lung tissues were stained with H&E using standard procedures (100x). No differences were noted at days 1 and 3 (not shown) but by days 7 or 14, changes in lung structure were evident and quantitative assessments of the changes are demonstrated in Figure 2. B) Morphometric analyses of lung tissue samples obtained from mouse pups exposed to 85% or >95% O2 or RA. Number of air spaces [1], number of secondary crests [2], airspace area [3], airspace perimeters [4], and septal thicknesses [5] were measured. Each data point represents tissues taken from three individual animals and at least five fields per slide were measured and averaged. Data were expressed as means ± SD and assessed by two-way ANOVA with modified t-test post hoc. An effect of exposure and an interaction were identified in number of alveoli, alveolar area, and alveolar perimeter, an effect of day, exposure, and an interaction were identified in number of alveolar crests and septal thickness. *different between RA and O2 exposed animals on the same day, §different from day 1 same treatment, ¶different between 85% and >95% O2 same day.

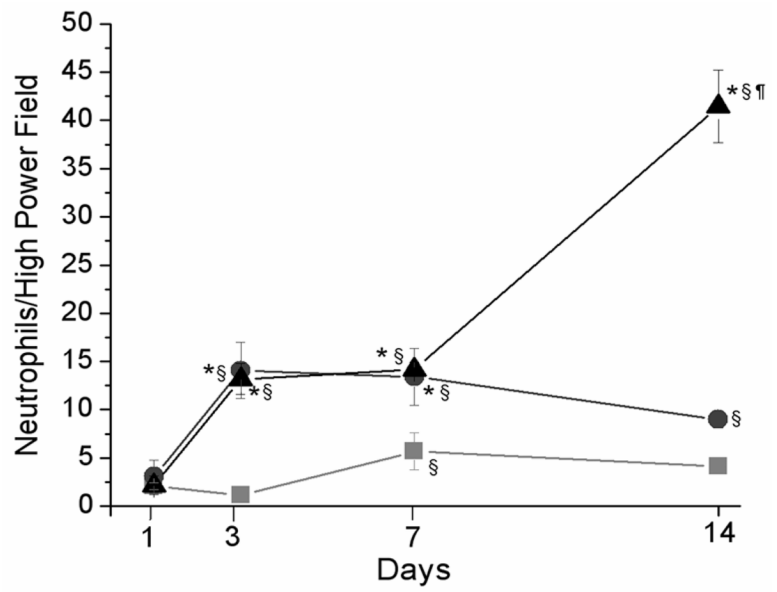

Figure 4.

Neutrophil accumulation in lung tissues of mice pups exposed 85% (▲) or >95% O2 (●), or RA (■). Pups were treated as described in Methods and fixed lung tissues were stained with anti-neutrophil antibodies. Four high power fields were counted for each section and three animals per treatment group. Graphs indicate means ± SD. Data were analyzed by two-way ANOVA with modified t-test post hoc. An effect of day and exposure and an interaction were observed. *different between RA and O2 exposed animals on the same day, § different from day 1 same treatment, ¶different between 85% and >95% O2 same day.

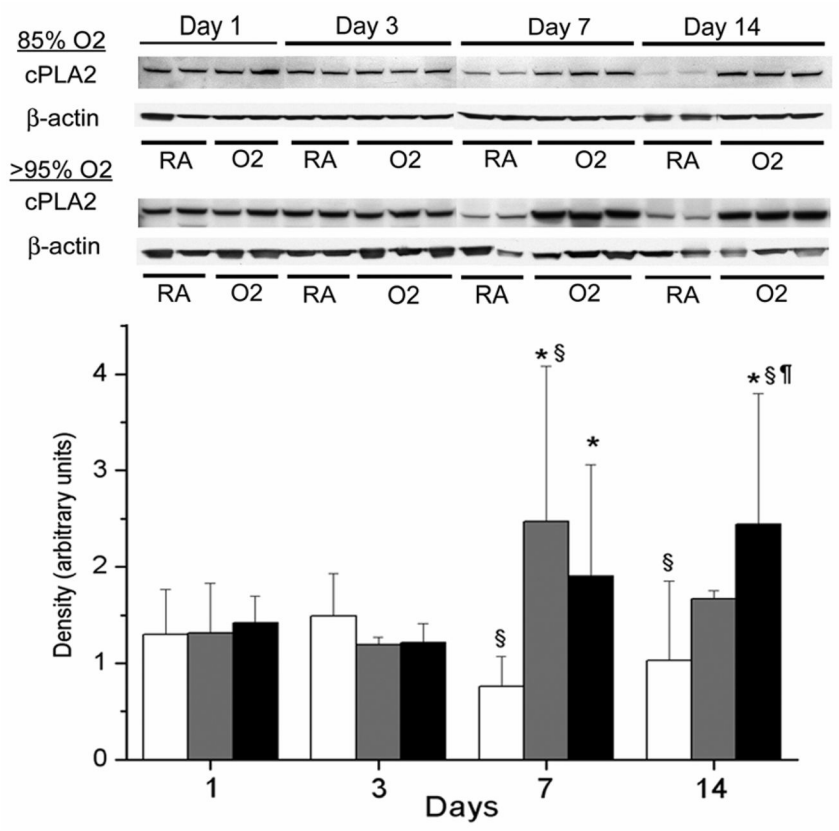

Figure 5.

CPLA2 levels in lung tissues of mouse pups exposed to 85% (▩) or >95% O2 (■), or RA (□). Pups were treated as described in Methods and proteins were quantitated by Western blot. CPLA2 band intensity was normalized to α-actin, and the ratios were analyzed by two-way ANOVA with modified t-test post hoc. Graphs indicate means ± SD, n=5–18. An effect of exposure and an interaction were observed. *different between RA and O2 exposed animals on the same day, §different from day 1 same treatment, ¶different between 85% and >95% O2 same day.

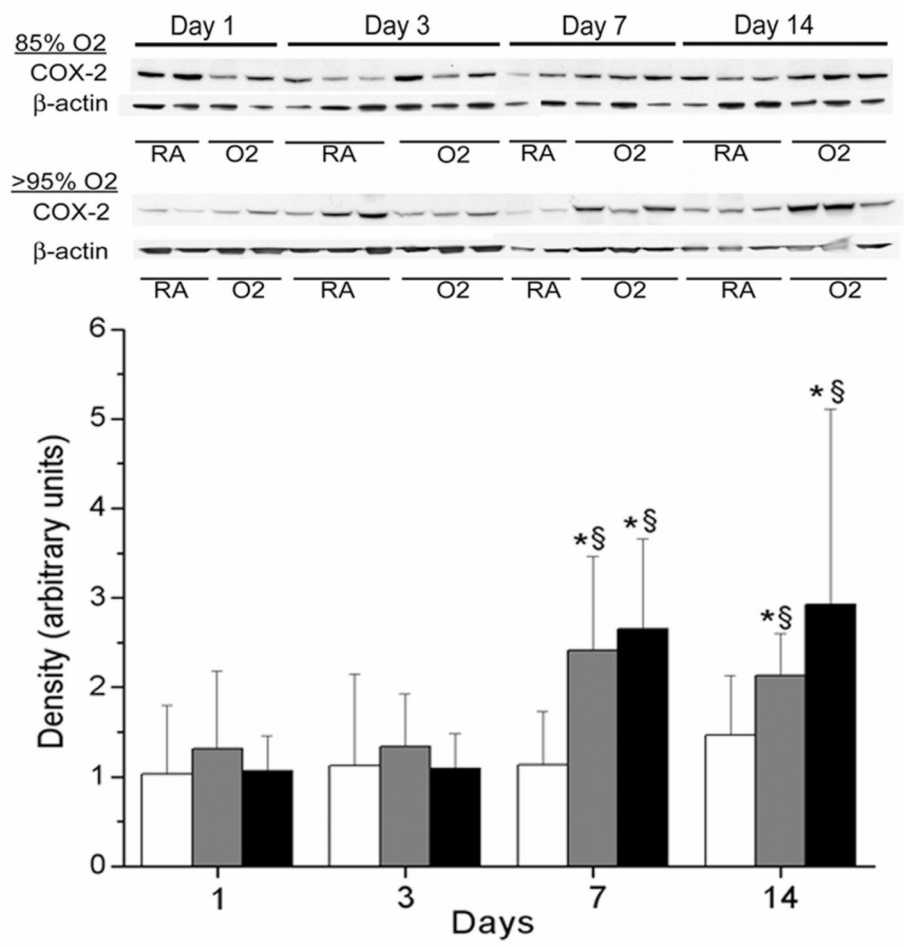

Figure 6.

COX-2 levels in lung tissues of mouse pups exposed to 85% (▩) or >95% O2 (■), or RA (□). Pups were treated as described in Methods and proteins were quantitated by Western blot. COX-2 band intensity was normalized to α-actin, and the ratios were analyzed by two-way ANOVA with modified t-test post hoc. Graphs indicate means ± SD, n=5–18. An effect of exposure and an effect of day were observed. *different between RA and O2 exposed animals on the same day, §different from day 1 same treatment, ¶different between 85% and >95% O2 same day.

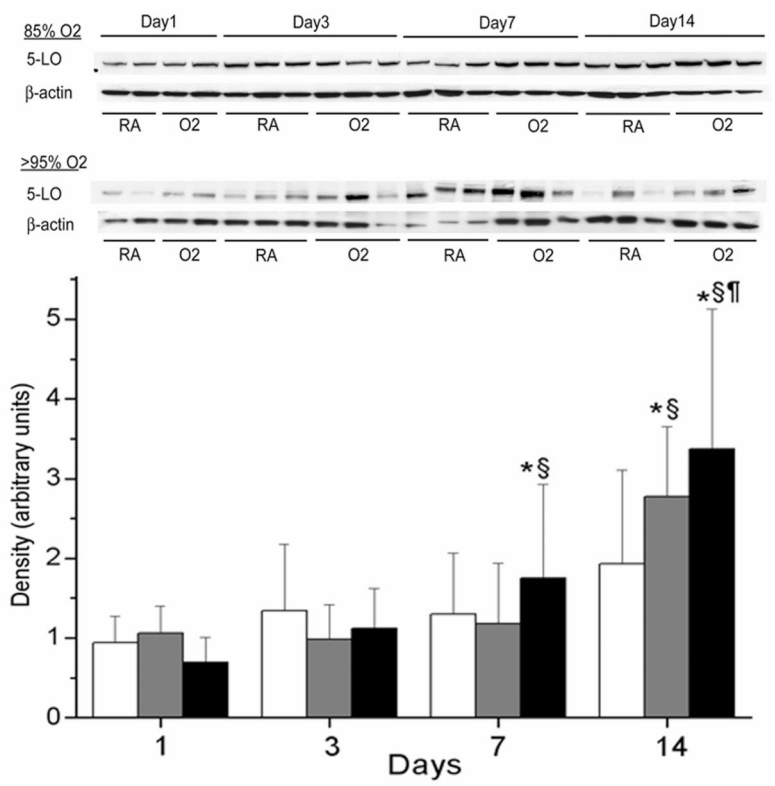

Figure 7.

5-LO levels in lung tissues of mouse pups exposed to 85% (▩) or >95% O2 (■), or RA (□). Pups were treated as described in Methods and proteins were quantitated by Western blot. 5-LO band intensity was normalized to α-actin, and the ratios were analyzed by two-way ANOVA with modified t-test post hoc. Graphs indicate means ± SD, n=5–18. An effect of exposure, an effect of day and an interaction between day and exposure were observed. *different between RA and O2 exposed animals on the same day, §different from day 1 same treatment, ¶different between 85% and >95% O2 same day.

Table 1

Eicosanoid levels in lung tissues of mouse pups exposed to RA, 85% or >95% O2.

		PGD_2	PGE_2	$PGF_{2\alpha}$	$TBXB_2$	5-HETE
Day 1	RA	0.71±0.13	1.93±0.35	2.15±0.12	0.30±0.04	0.85±0.13
	85%	1.55±0.13*	3.72±0.38	2.93±0.28*	0.44±0.09	0.88±0.26
	>95%	0.82±0.11	1.98±0.17	2.22±0.12¶	0.32±0.02	0.79±0.11
Day 3	RA	0.58±0.14	1.47±0.47	1.78±0.18	0.28±0.44	0.69±0.06
	85%	0.94±0.21	2.59±0.89	2.18±0.17§	0.52±0.15	0.96±0.29*
	>95%	0.49±0.44	1.10±0.11	1.65±0.22¶§	0.17±0.08	0.60±0.13
Day 7	RA	1.16±0.16	3.23±0.60	2.11±0.25	1.17±0.26§	0.42±0.05§
	85%	1.25±0.45	5.48±1.57	2.02±0.13§	1.63±0.55§	0.65±0.16
	>95%	4.08±0.87*¶	8.94±2.16*¶§	3.75±0.57*¶§	1.69±0.60*§	0.63±0.12
Day 14	RA	0.93±0.15	4.16±0.68	2.43±0.28	1.11±0.15§	0.35±0.05§
	85%	2.16±1.19*§	3.48±0.60	2.74±0.31	0.67±0.11	0.29±0.03§
	>95%	2.49±0.49*§	6.84±1.36§	2.36±0.10	1.21±0.09§	0.39±0.05§

Data are expressed as pmol/g tissue.

Lung tissues obtained from C3H/HeN mouse pups were homogenized and the lipids were extracted and analyzed by LC-MS/MS (described in Methods). Data are expressed as mean ± SD, and analyzed by two-way ANOVA, with modified t-test post hoc for individual differences, $p<0.05$, n=5–7. An effect of day, an effect of exposure and an interaction between day and exposure were identified for PGD_2, an effect of day and an interaction between day and exposure were identified $PGF_{2\alpha}$, an effect of day was identified for PGE_2, $TBXB_2$, and 5-HETE.

*indicates different from same day RA exposure,

§indicate different from day 1 same exposure,

¶indicates differences between 85% and >95% O2 same day.