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Timing of hyperoxic exposure during alveolarization influences damage mediated by leukotrienes

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Departments of 1Physiology, 2Pediatrics, and 3Obstetrics and Gynecology and Perinatal Research Centre, University of Alberta, Edmonton, Alberta T6G 2S2, Canada

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Manji, Jacqueline S., Cian J. O’Kelly, Wynne I. Leung, and David M. Olson. Timing of hyperoxic exposure during alveolarization influences damage mediated by leukotrienes. Am J Physiol Lung Cell Mol Physiol 281: L799–L806, 2001.—Hyperoxic exposure of rat pups during alveolarization (postnatal days 4–14) severely retards alveolar development. Some aspects of this inhibition are mediated by leukotrienes (LTs) and may be time sensitive. We determined 1) the effects of exposure to hyperoxia (O2) during discrete periods before and during alveolarization on developing alveoli and 2) whether a relationship exists between O2 and LTs in these periods. Pups were exposed to >95% O2 from days 1 to 4, 4 to 9, 9 to 14, or 4 to 14 in the absence and presence of the LT synthesis inhibitor MK-0591. Both the level of in vitro lung tissue LT output on days 4, 9, and 14 and the degree of alveolarization on day 14 were determined. Pups exposed to O2 from days 4 to 9 had a more profound inhibition of alveolarization on day 14 compared with those exposed to O2 from days 1 to 4 or 9 to 14. Peptido-LT levels were significantly higher in pups exposed to O2 on days 9 and 14 compared with pups in air and returned to normal once normoxia was restored. LT inhibition from days 4 to 14, 4 to 9, or 9 to 14 in pups exposed to O2 from days 4 to 14 prevented the O2-induced inhibition of alveolarization. These data suggest that developing alveoli are sensitive to LTs shortly before and after day 9, significantly retarding certain parameters of alveolarization on day 14. We conclude that some of the effects of O2 are not uniform throughout different stages of alveolarization and that this is likely related to the timing of LT exposure.

MK-0591; morphometry; 5-lipoxygenase-activating protein

HYPEROXIA CAN SEVERELY DAMAGE the developing lung by preventing septation of sacculles into alveoli (24). This leads to a decrease in the gas-exchange surface area. Our laboratory (2) has previously shown that the leukotrienes (LTs) produced from arachidonic acid by 5-lipoxygenase and the 5-lipoxygenase-activating protein (FLAP) are involved in mediating this damage. Rat pups exposed to hyperoxia from postnatal days 4 to 14 [the period when most of the alveolarization in the rat occurs (7)] showed elevated LT levels on day 14. This correlated with marked inhibition of alveolar development. Inhibition of LT synthesis with the FLAP inhib-
Animal Service of the University of Alberta under veterinary care. The guidelines of the Canadian Council of Animal Care were followed in all experimental procedures. Dams were maintained on regular laboratory rodent pellets and water ad libitum and kept on a 12:12-h light-dark cycle.

**O\textsubscript{2} Exposure and LT Inhibition**

Parallel litters of randomly divided rat pups and their dams were placed in 0.14-m\textsuperscript{3} Plexiglas chambers and maintained in hyperoxic or normoxic conditions. \(O\textsubscript{2}\) concentrations were monitored daily (\(O\textsubscript{2}\) analyzer 5517, Ventronics, Temecula, CA). \(O\textsubscript{2}\) and air were filtered through barium hydroxide lime (Baralyme; Allied Healthcare Products, St. Louis, MO) to keep \(CO\textsubscript{2}\) and humidity were maintained at 26°C and 75–80%, respectively. Chambers were opened for 15 min daily to switch dams between cages and administer drugs if required.

Pups were maintained in >95 or 21% \(O\textsubscript{2}\) from days 1 to 4, 4 to 9, 9 to 14, or 4 to 14 after birth. In another experiment, pups were exposed to >95 or 21% \(O\textsubscript{2}\) from days 4 to 14, and MK-0591 [20 mg/kg given subcutaneously (sc) once daily; Merck Frosst, Dorval, PQ] was administered from days 3 to 9, 9 to 14, or 3 to 14. MK-0591 is a FLAP inhibitor that has been shown (3, 12, 23) to be a potent inhibitor of LT production. Controls for MK-0591 were different animals within the same litter injected with the vehicle for the drug (\(H\textsubscript{2}O\) plus Tween 80 in a 4:1 ratio).

**Peptido-LT Production**

Lung peptido-LT production was measured with a short-duration lung explant technique. Briefly, pups were killed with an overdose of pentobarbital sodium (100 mg/kg of Euthanyl; MTC Pharmaceuticals, Cambridge, ON). Three 500-μm-thick blood-free lung slices (sliced with a tissue slicer from Stoelting, Wood Dale, IL) were placed in tissue culture wells (12-well plate; Costar, Cambridge, MA) containing 800 μl of culture medium (Hanks’ balanced salt solution with 10% fetal bovine serum, HEPES, pH 7.36, and 1.67 mM CaCl\textsubscript{2}) and were incubated at 37°C for 30 min. Culture medium was stored at −70°C before being assayed for LT levels with ELISA.

Peptido-LTs were assayed with LTC\textsubscript{4}, LTD\textsubscript{4}, and LTE\textsubscript{4}. ELISA kits from Oxford Biomedical Research or Cayman Chemical.

Typical results for the Oxford kits yield 50% of the sample or standard bound to medium bound at 1.93 ng/ml and 80% standard bound to medium bound at 0.83 ng/ml. The specificity of the kits used was 100% for LTC\textsubscript{4}, >80% for LTD\textsubscript{4}, and LTE\textsubscript{4}, <2% for LTA\textsubscript{4}, and <1% for LTB\textsubscript{4}. The peptido-LT levels were normalized by the total DNA [assay modified from Downs and Willfinger (13)] contained in the lung tissue.

**Lung Morphometry**

Lung preparation. Lungs were fixed in situ through a polyethylene tracheal cannula with 2.5% glutaraldehyde at a constant pressure of 20 cm\(H\textsubscript{2}O\) for 2 h. Then the trachea was ligated, and the lungs were excised and immersed in glutaraldehyde for 24 h. Lung volumes were measured by water displacement before and after the 24-h fixation period to detect shrinkage. Because of minimal shrinkage (0–2%), the data were not corrected.

After fixation, transverse sections of superior, middle, and inferior portions of right lung and superior and inferior portions of left lung were embedded in paraffin. The entire transverse sections were cut 3 μm thick and stained with Gomori-trichromaldehyde fuscin. Slides were initially examined to eliminate sections with evidence of inadequate preparation.

Parenchymal morphometry. Light-level morphometric assessment of lung parenchymal tissue was performed in a blinded fashion on coded slides from 6–15 animals/experimental group. Ten randomly selected fields were examined from each lung. Histological specimens observed with the microscope (Carl Zeiss Jenamed Variant) were put in a gray image analyzer system (IS Tech) via a video camera (MTI S 68). The measurements and calculations were performed with Genias 25 image analysis software (Joyce-Loebl).

Parenchymal tissue includes alveolar septa, alveolar ducts, respiratory bronchiolar tissues, and blood vessels with a diameter ≤ 10 μm and their contents. Volume density of parenchymal tissue (\(V_p\)) was calculated as field area (FA) – airspace area (\(A_{spec}\))/FA × 100 from each analyzed field. Mean septal thickness (\(T_{sept}\)) was calculated from the parenchymal tissue area and the length of the gas-exchange surface.

As an indication of mean alveolar diameter, the mean linear chord length (\(L_m\)) was calculated by dividing the length of the computer-generated horizontal test line by the number of intercepts of the septal wall. Mean volume of airspace units (\(V_{spec}\)) was computed as \((L_m/\pi)\). To detect the structural changes in alveolar airspaces (i.e., the shape), the airspace perimeter-to-airspace area ratio (\(P/A\)) was calculated from each field. \(P/A\) gives an indication of the shape of the alveoli. A lower ratio indicates a simple, more rounded structure (i.e., less septum protruding into the airspace).

The internal surface area of the lung available for respiratory exchange was calculated with the formula \(4 \times \text{lung volume}/L_m\). These data were normalized to 100 g of body weight and used as specific internal surface area (SISA).

**Statistical Analyses**

The data were analyzed with Student’s \(t\)-test or analysis of variance when the variation was distributed according to treatment and time. Duncan’s new multiple range post hoc test was used to determine differences between groups when a significant \(F\)-value was obtained. Significance was achieved at \(P \leq 0.05\). Data are expressed as means ± SD.

**RESULTS**

**LT Production**

\(O\textsubscript{2}\) exposure from days 1 to 4 caused a significant increase in peptido-LT production on day 4 compared with air-exposed control lungs. These levels returned to normal by days 9 and 14 (Fig. 1). \(O\textsubscript{2}\) exposure from days 4 to 9 caused a significant (\(P < 0.05\)) increase in the amount of peptido-LT produced by rat pup lungs measured on day 9 compared with that in air-exposed control lungs (Fig. 2). By day 14, peptido-LT returned to control levels in these pups. However, on day 14, pups exposed to \(O\textsubscript{2}\) from days 4 to 14 and 9 to 14 produced significantly (\(P < 0.05\)) higher levels of peptido-LTs than air-exposed control pups (Fig. 2).

Exposure to \(O\textsubscript{2}\) from days 4 to 9 caused a significant increase in LTs produced by the lung on day 9; this was significantly reduced by MK-0591 (Fig. 3). Hyperoxia treatment from days 4 to 14 in vivo significantly increased peptido-LT output on day 14 by lung slices in vitro; only MK-0591 treatment from days 9 to 14 or 3 to
14, but not from days 3 to 9, inhibited this increase (Fig. 3).

Morphometry

Effects of differential exposure to hyperoxia. Representative photomicrographs of lung parenchyma from each of the experimental groups are depicted in Fig. 4. Compared with air-exposed animals, the rats exposed to hyperoxia from days 4 to 14 had larger and more simplified alveolar airspaces. The animals exposed on days 4–9 exhibited parallel changes in alveolar structure, although to a lesser extent than the changes noted in the day 4–14 group. In contrast, results from the day 9–14 animals were similar to those of the air-exposed animals with respect to alveolar size and shape.

These visual impressions are correlated and quantified by the morphometric data (Table 1). Exposure to >95% O₂ from days 4 to 14 caused an increase in the calculated V\text{aspunit}. The pups exposed from days 4 to 9 manifested elevated V\text{aspunit} compared with air-exposed animals. However, the increases seen in this group were significantly lower than those observed for the day 4–14 group. Four-day exposure before day 4 and after day 9 had no detectable effect on this alveolar size-related parameter. The intergroup differences seen with respect to V\text{aspunit} are mirrored by the P/A. The animals exposed from days 4 to 14 showed evidence of simplified alveoli as indicated by a decreased P/A. The day 4–9 group also had a decreased P/A, but again, this decrease was not as great as that associated with the day 4–14 group. Animals exposed from days 9 to 14 had a P/A comparable to that of the air-exposed animals.

In contrast with the parameters described above, significant alterations in SISA were only seen in the group exposed from days 4 to 14. The pups in this group had a decreased lung surface area.

Some different trends were observable with respect to measures of parenchymal tissue and septal thickness. The relative amount of lung parenchymal tissue, as assessed by V\text{p}, was similarly reduced compared with values from air-exposed animals in the day 4–9 and 4–14 groups. Hyperoxia from days 9 to 14 resulted in lower, but not significantly different, V\text{p} values. Meanwhile, T\text{sept} was significantly lower than that in air-exposed animals in the day 4–9 and 9–14 groups.

Fig. 2. Effects of >95% O₂ exposure from postnatal days 1 to 4 on rat lung tissue peptido-leukotriene (peptido-LT) output as measured on days 4, 9, and 14. Data are means ± SD; n = 6 lungs/group. O₂ exposure from days 1 to 4 significantly stimulated peptido-LT production in 4-day-old rats (P < 0.05). Removal from O₂ allowed peptido-LT levels to return to normal as measured at 9 and 14 days of age. Values that do not share a common letter are significantly different from one another (P < 0.05).

Fig. 1. Effects of exposure to >95% O₂ from postnatal days 1 to 4 on rat lung tissue peptido-leukotriene (peptido-LT) output as measured on days 4, 9, and 14. Data are means ± SD; n = 6 lungs/group. O₂ exposure from days 1 to 4 significantly stimulated peptido-LT production in 4-day-old rats (P < 0.05). Removal from O₂ allowed peptido-LT levels to return to normal as measured at 9 and 14 days of age. Values that do not share a common letter are significantly different from one another (P < 0.05).
Fig. 3. Effects of >95% O2 exposure from d4–14 with and without MK-0591 (20 mg/kg given subcutaneously once daily) from d3–9, d9–14, or d3–14 on rat lung tissue peptido-LT output measured on days 9 (A) and 14 (B). Data are means ± SD; n = 6 lungs/group. O2 exposure from d4–14 significantly (P < 0.05) increased peptido-LT production on days 9 and 14. MK-0591 administration from d3–9 significantly (P < 0.05) inhibited peptido-LT production on day 9 in O2-exposed pups, a condition that reversed by day 14. MK-0591 administration from d3–14 or d9–14 significantly (P < 0.05) inhibited peptido-LT production on day 14. Values that do not share a common letter are significantly different from one another (P < 0.05).

The day 4–14 animals were transitional between the air-exposed animals and these two hyperoxic groups, showing no significant differences compared with air-exposed animals but also no significant differences compared with the day 4–9 and 9–14 groups.

Effects of LT synthesis inhibition. In Fig. 5, photomicrographs show 14-day-old rat pups exposed to 21% O2 plus vehicle (A) or >95% O2 plus vehicle from days 4 to 14 (B), along with administration of MK-0591 (20 mg/kg sc once daily) from days 4 to 9 or 9 to 14. It is obvious from these photomicrographs that O2 plus vehicle animals have much larger and more simplified alveoli than air plus vehicle animals. This study confirmed a previous study (2) that found that the administration of MK-0591 from days 3 to 14 prevented damage caused by hyperoxia from days 4 to 14 (data not shown). Also evident from these photomicrographs is the prevention of this event in hyperoxic animals given MK-0591 from days 4 to 9 (Fig. 5C) or 9 to 14 (Fig. 5D), whose alveolar structure was similar to that of the air plus vehicle group. The drug had no effect in air-exposed animals (data not shown).

The comparative lung morphometric findings for MK-0591- or vehicle-treated air and hyperoxic pups on day 14 are shown in Table 2. The Vp of O2 plus vehicle pups was significantly lower than that of all air-exposed groups as well as all three O2 plus MK-0591 groups. Alveolar septa were significantly thicker in all hyperoxia-exposed animals regardless of MK-0591 administration compared with the air-exposed animals, except for the air plus MK-0591 (day 9–14) group, which was statistically similar. As with previous studies (2), hyperoxia alone had a significant alveolar-enlarging effect, as indicated by Vaspunit, compared with that in air-exposed animals. The Vaspunit was not significantly different in hyperoxia-exposed pups administered MK-0591 at any time compared with air-exposed pups. The P/A was significantly smaller in the O2 plus vehicle group compared with that in all air-exposed groups. Statistical analysis revealed that although the P/A values of all three of the O2 plus MK-0591 groups were not significantly different from those in the air-exposed groups, they were also not significantly different from the O2 plus vehicle group. The P/A trend was toward an increase in the O2 plus MK-0591 group compared with the O2 plus vehicle groups, making them more like the air plus vehicle groups. A decrease was observed in SISA development in O2 plus vehicle pups compared with air-exposed pups that was not observed in the O2 plus MK-0591 pups (day 3–9 or O2 plus MK-0591 (day 9–14) groups. Although SISA was higher in the O2 plus MK-0591 (day 3–14) pups than in the control O2 plus vehicle pups, it did not reach statistical significance (P = 0.07).

DISCUSSION

Hyperoxic exposure upsets the normal cellular oxidant-antioxidant defense equilibrium by producing marked increases in O2 free radical production (30). Prolonged exposure of newborn and adult rats to high levels of O2 causes lung damage characterized by interstitial and intra-alveolar edema followed by infiltration of protein, entry of cells, and, finally, hemorrhage into the alveolar space (9). Inhibition of the process of alveolarization is also a hallmark of hyperoxic exposure during the newborn period (4, 21, 29).

Hyperoxia has a major impact on lung development. It slows lung maturation and causes a reduction in alveolar number and surface area by permanently inhibiting the process of septation (21). Saccule septation is permanently diminished and the parenchymal airspace is enlarged, with irregular dilatation of alveoli and alveolar ducts, conditions that exist until adulthood in rats (24). Decreased cell proliferation is a well-known concomitance of hyperoxic exposure in the
newborn lung; however, certain cell types, including alveolar type II cells, proliferate when exposed to high levels of O$_2$ (13a). Because the process of septation undoubtedly involves a very coordinated proliferation and, perhaps, apoptosis of specific cell types, a factor that can inhibit DNA synthesis or induce gene expression (such as hyperoxia) can easily disrupt this developmental process of the lung (17).

In our model of newborn hyperoxic damage, 10 days of exposure to $>$95% O$_2$ caused marked interstitial and intra-alveolar edema and proteinosis in newborn rats (2). Altered alveolarization occurred in hyperoxic animals, leading to increases in V$_{asp}$ units, which corresponds to the results of other studies cited above.

The timing of exposure to O$_2$ is very important. Hyperoxic exposure after the lung has gone through its developmental phase has different effects than exposure during the period of alveolarization (8, 17, 27). In fact, in the rat, the period of alveolarization (postnatal days 4–14) has been defined as a critical period of lung development, during which time any stress to normal development, including O$_2$, can have profound long-lasting effects (21).

Hyperoxic exposure at different time periods during alveolarization was investigated in this study as a means of further describing how hyperoxia affects the developing lung. Exposure to $>$95% O$_2$ from days 1 to 4 caused more profound changes in alveolarization (i.e., larger V$_{asp}$ units, smaller P/A), as measured on day 14, than did exposure from days 9 to 14 or 1 to 4. However, exposure during these other time periods had some impact. Exposure from days 9 to 14 led to

![Fig. 4. Photomicrographs of lung parenchyma of 14-day-old rats exposed to 21% O$_2$ (A), $>$95% O$_2$ from days 1 to 4 (B), $>$95% O$_2$ from days 4 to 9 (C), $>$95% O$_2$ from days 9 to 14 (D), and $>$95% O$_2$ from days 4 to 14 (E). Parenchymal architecture of hyperoxic lungs exposed from days 4 to 9 and days 4 to 14 is simpler (fewer and enlarged alveoli; C and E). Hyperoxic exposure from days 1 to 4 and 9 to 14 had little effect. Bars, 100 μm.](image)

### Table 1. Lung morphometry of 14-day-old rat pups exposed to $>$95% O$_2$

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>V$_{asp}$, μm$^2 \times 10^5$</th>
<th>P/A</th>
<th>V$_p$, %</th>
<th>T$_{sept}$, μm</th>
<th>SISA, cm$^2$ g$^{-1}$ × 10$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>13</td>
<td>0.73 ± 0.21$^*$</td>
<td>1.04 ± 0.10$^*$</td>
<td>33.7 ± 3.6$^*$</td>
<td>0.99 ± 0.12$^*$</td>
<td>4.89 ± 0.66$^*$</td>
</tr>
<tr>
<td>O$_2$ (1–4)</td>
<td>6</td>
<td>0.68 ± 0.14$^*$</td>
<td>1.11 ± 0.05$^*$</td>
<td>38.0 ± 1.8$^*$</td>
<td>1.11 ± 0.07$^*$</td>
<td>4.84 ± 0.35$^*$</td>
</tr>
<tr>
<td>O$_2$ (4–9)</td>
<td>15</td>
<td>1.03 ± 0.33$^*$</td>
<td>0.96 ± 0.11$^*$</td>
<td>29.6 ± 5.4$^*$</td>
<td>0.89 ± 0.16$^*$</td>
<td>4.81 ± 0.69$^*$</td>
</tr>
<tr>
<td>O$_2$ (9–14)</td>
<td>14</td>
<td>0.63 ± 0.15$^*$</td>
<td>1.07 ± 0.06$^*$</td>
<td>30.5 ± 2.3$^*$</td>
<td>0.85 ± 0.11$^*$</td>
<td>5.26 ± 0.65$^*$</td>
</tr>
<tr>
<td>O$_2$ (4–14)</td>
<td>6</td>
<td>1.30 ± 0.18$^*$</td>
<td>0.80 ± 0.07$^*$</td>
<td>27.1 ± 1.3$^*$</td>
<td>0.93 ± 0.04$^*$</td>
<td>4.16 ± 0.37$^*$</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats; nos. in parentheses, postnatal days. V$_{asp}$, airspace unit volume; T$_{sept}$, septal thickness; SISA, specific internal surface area; V$_p$, volume density of parenchymal tissue; P/A, ratio of airspace perimeter to area. Within each morphometric parameter, values that do not share a common symbol are significantly different from one another ($P < 0.05$).
significant septal thinning, whereas animals exposed from days 1 to 4 had thicker septa on day 14. Thus the effects of hyperoxic exposure are highly dependent on the timing of the exposure. In addition, the timing of the assessment of lung morphology may be important in determining the influence of a critical period of hyperoxia on development. Our determinations all took place on day 14. This was appropriate for the day 1–4 and 4–9 groups, but it may have been too early to detect effects resulting from hyperoxia in the day 9–14 group. Indeed, Blanco and Frank (1) exposed newborn rats to >95% O₂ from days 18 to 28 and determined on day 28 that hyperoxia during this period also inhibited septation, suggesting that the response may require more than 5 days to become evident. Thus the data show that hyperoxia during days 1–4 does not meaningfully inhibit alveolarization, that exposure during days 4–9 definitely retards alveolarization, and that alveolarization is largely normal on day 14 when pups are exposed on days 9–14. Hence one critical period for O₂ exposure exists from days 4 to 9.

Several investigators have suggested that the eicosanoids produced by inflammatory cells or by cells lining the alveoli may be involved in hyperoxic lung damage. Koyanagi (19) showed that exposure to hyperoxia from day 1 leads to marked increases in production of LTB₄ by the lung. Taniguchi et al. (26) found an increase in LTB₄ levels in the bronchoalveolar lavage fluid of adult rats exposed to 85% O₂ for 60 h. Elevated LT concentrations have been found in the bronchoalveolar lavage fluid of infants with bronchopulmonary dysplasia, a disease for which hyperoxia is a known pathogenic factor (14, 25). LTB₄ is a well-known chemottractant for neutrophils, and the activated inflammatory cells can release other radicals and mediators that may damage normal tissue (27). It is known that LTC₄ and LTD₄ increase vascular permeability, and it has been shown that LTs can provoke pressor responses and edema in perfused rat lungs (11, 16).

Several investigators have detected increased LT levels due to oxidative stress. Burghuber et al. (5) demonstrated an increased 5-hydroxyeicosatetraenoic acid production and edema of perfused isolated rat lungs caused by glucose oxidase that was diminished by LT synthesis inhibitors. Similarly, increased LTD₄ and LTB₄ were shown in isolated rabbit lungs infused with tertiary butyl peroxide (15). The accompanying increase in vascular permeability was ameliorated by the LT antagonist FPL-55712.

We found that lung explants exposed to >95% O₂ in vivo displayed increases in peptido-LT output in vitro that returned to normal once normoxic conditions were restored.

Table 2. Lung morphometry of 14-day-old rat pups exposed to >95% O₂ with and without MK-0591

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Vₐlveolar, µm² × 10⁵</th>
<th>P/A</th>
<th>Vᵥ, %</th>
<th>Tᵩₑᵦ, µm</th>
<th>SISA, cm²/g × 10³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air + vehicle</td>
<td>10</td>
<td>0.56 ± 0.27*</td>
<td>0.94 ± 0.10*</td>
<td>24.1 ± 2.3</td>
<td>0.67 ± 0.03</td>
<td>5.97 ± 1.39</td>
</tr>
<tr>
<td>Air + MK-0591 (3–9)</td>
<td>6</td>
<td>0.65 ± 0.23*</td>
<td>0.96 ± 0.04*</td>
<td>25.5 ± 1.7</td>
<td>0.71 ± 0.07</td>
<td>6.10 ± 1.03</td>
</tr>
<tr>
<td>Air + MK-0591 (9–14)</td>
<td>6</td>
<td>0.63 ± 0.14*</td>
<td>1.01 ± 0.06†</td>
<td>25.5 ± 2.9</td>
<td>0.74 ± 0.08†</td>
<td>5.94 ± 0.90</td>
</tr>
<tr>
<td>Air + MK-0591 (3–14)</td>
<td>7</td>
<td>0.79 ± 0.29*</td>
<td>0.90 ± 0.10*</td>
<td>24.5 ± 2.5</td>
<td>0.69 ± 0.07*</td>
<td>5.05 ± 0.49</td>
</tr>
<tr>
<td>O₂ + vehicle</td>
<td>12</td>
<td>1.28 ± 0.54†</td>
<td>0.82 ± 0.12†</td>
<td>21.5 ± 3.6</td>
<td>0.72 ± 0.06</td>
<td>4.56 ± 0.52</td>
</tr>
<tr>
<td>O₂ + MK-0591 (3–9)</td>
<td>8</td>
<td>0.89 ± 0.33*</td>
<td>0.91 ± 0.10†</td>
<td>25.8 ± 3.1</td>
<td>0.78 ± 0.07†</td>
<td>5.66 ± 0.79</td>
</tr>
<tr>
<td>O₂ + MK-0591 (9–14)</td>
<td>7</td>
<td>0.74 ± 0.26*</td>
<td>0.85 ± 0.04†</td>
<td>24.5 ± 1.8</td>
<td>0.74 ± 0.04</td>
<td>5.13 ± 0.83</td>
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<tr>
<td>O₂ + MK-0591 (3–14)</td>
<td>8</td>
<td>0.80 ± 0.26*</td>
<td>0.89 ± 0.07†</td>
<td>24.6 ± 2.1</td>
<td>0.73 ± 0.06</td>
<td>4.94 ± 0.36</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats; nos. in parentheses, postnatal days. MK-0591 (20 mg/kg) was given subcutaneously once daily. Within each morphometric parameter, values that do not share a common symbol are significantly different from one another (P < 0.05).
restored, a necessary prerequisite for a mediator. To test further the possibility that LTs might mediate the effect of hyperoxia, inhibition of LT synthesis was achieved by administration of MK-0591. Our expectation was that LT inhibition during the second half of hyperoxia, days 9–14, would not lead to improvement of alveolar development in animals exposed to hyperoxia from days 4 to 14 because the damage would have occurred during the first half of the hyperoxic exposure. Instead, administration of MK-0591 from days 4 to 14, 4 to 9, and 9 to 14 led to alveolar improvement on day 14, especially in mean V_{\text{A}}/\text{V_{p}}, \text{V}_{\text{p}}, and SISA. We interpret these data to suggest that LTs do mediate some of the effects of hyperoxia on alveolarization but that a lag period exists between exposure to hyperoxia and the production of LTs and the subsequent removal from the hyperoxic stimulus and the decrease of LT production. Recent data from our laboratory (Hosford GE, unpublished observations) indicates that exposure to hyperoxia does increase the immunoreactive protein mass of 5-lipoxygenase and FLAP after ~4 days of exposure. These data suggest, therefore, that if a critical period of sensitivity to LTs exists, it appears to be toward the middle stages of alveolarization. This is evident from the facts that 1) hyperoxia during days 1–4 increases LTs that do not inhibit alveolarization; 2) inhibition of alveolarization results from increases in LTs caused by hyperoxia before day 9, a process that continues after normoxic conditions are reestablished; 3) inhibiting the hyperoxia-induced synthesis of LTs only up to day 9 permits mostly normal alveolarization; and 4) inhibiting production of LTs from day 9 also permits mostly normal alveolarization.

The mechanisms that lead to inhibited septation are not defined, but the literature would suggest that either inhibition of cell proliferation or premature sepal thinning may be factors that have a role. In our study, the very uniform improvement of only certain alveolar parameters is consistent with a LT inhibition of cell proliferation and not septal thinning, a process that requires apoptosis. The very rapid proliferation of epithelial, fibroblast, and endothelial cells in the developing alveoli during days 4–10 in the newborn rat suggests strongly that any inhibition of this growth would lead to altered alveolarization (18). Tschanz et al. (28) suggested that glucocorticoids may prevent septation by premature sepal thinning before septa erupt and form a double-capillary network. Our findings that hyperoxia from days 4 to 9 or days 9 to 14 caused sepal thinning suggest a role for O2 in this process but one that is not linked to LTs because the LT synthesis inhibitor had no effect on thinning.

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