Oxygen-dependent Lipid Peroxidation during Lung Ischemia

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Abstract

The effect of alveolar oxygen tension on lung lipid peroxidation during lung ischemia was evaluated by using isolated rat lungs perfused with synthetic medium. After a 5-min equilibration period, global ischemia was produced by discontinuing perfusion while ventilation continued with gas mixtures containing 5% CO_2 and a fixed oxygen concentration between 0 and 95%. Lipid peroxidation was assessed by measurement of tissue thiobarbituric acid–reactive products and conjugated dienes. Control studies (no ischemia) showed no change in parameters of lipid peroxidation during 1 h of perfusion and ventilation with 20% or 95% O_2. With 60 min of ischemia, there was increased lipid peroxidation which varied with oxygen content of the ventilating gas and was markedly inhibited by ventilation with N_2. Perfusion with 5-, 8-, 11-, 14-eicosatetraynoic acid indicated that generation of eicosanoids during ischemia accounted for $\sim$ 40–50% of lung lipid peroxide production. Changes of CO_2 content of the ventilating gas (to alter tissue pH) or of perfusate glucose concentration had no effect on lipid peroxidation during ischemia, but perfusion at 8% of the normal flow rate prevented lipid peroxidation. Lung dry/wet weight measured after 3 min of reperfusion showed good correlation between lung fluid accumulation and lipid peroxidation. These results indicate that reperfusion is not necessary for lipid peroxidation with ischemic insult of the lung and provide evidence that elevated PO_2 during ischemia accelerates the rate of tissue injury. (*J. Clin. Invest.* 1991. 88:674–679.) Key words: conjugated dienes • hyperoxia • ischemia/reperfusion injury • malondialdehyde • perfused lung

Introduction

Recent studies have provided evidence that tissue damage can occur during the reperfusion period that follows an episode of ischemia (1–4). The mechanism for injury appears to be oxidative damage, in some cases because of activation of the xanthine oxidase enzyme (2) or other reactions for generation of oxidizing chemical species (3). This phenomenon of ischemia/

reperfusion injury has been described and studied extensively in the intestine, kidneys, heart, and brain, among other organs (4). In these organs, interruption of perfusion results in both ischemia as well as tissue hypoxia, and it is widely believed that the reintroduction of oxygen to the tissues plays a major role in the production of oxidative injury (1).

Ischemia/reperfusion injury has also been described in the lung, although the metabolic mechanisms have not yet been elucidated (5–10). It is important to note that unlike other organs, ischemia of the lung does not result necessarily in tissue hypoxia. In fact, cessation of pulmonary perfusion to a lung segment, assuming no change in ventilation, might be expected to increase alveolar PO_2 and oxygenation of the tissue in that segment. Therefore, reperfusion is not necessary for the "restoration" of tissue oxygenation with lung ischemia. As a corollary, the lung affords a unique opportunity to separate the effects of ischemia and anoxia on tissue function.

In this study, we investigated the effect of alveolar O_2 tension during the ischemic period on tissue oxidative damage, i.e., lipid peroxidation assessed by the measurement of thiobarbituric acid–reactive substances (TBARS)[1] and of conjugated dienes in the lung tissue. The experimental model was the isolated perfused rat lung where the alveolar PO_2 could be controlled by manipulation of the ventilating gas and lung ischemia could be produced by a global cessation of perfusion.

Methods

Sprague-Dawley male rats, CRL:CD (SD) BR (Charles River Breeding Laboratories, Kingston, NY) weighing 180–200 g, were anesthetized with pentobarbital, 50 mg/kg, and prepared for isolated lung perfusion using recirculating perfusate as previously described (11). The trachea was cannulated and lungs were ventilated at 60 cycles/min, 2 ml tidal volume, and 2 cm H_2O end-expiratory pressure. The thorax was then opened and a cannula was placed in the main pulmonary artery through the transected heart. The lungs were initially cleared of blood by gravity flow of perfusate (25 cm H_2O pressure), carefully removed from the thorax, trimmed of cardiac and other nonpulmonary tissue, and then transferred to the water-jacketed perfusion chamber maintained at 37°C. There was no interruption of ventilation during this transfer process and interruption of lung perfusion was < 5 s. Perfusion into the pulmonary artery was maintained by a peristaltic pump at a constant flow rate of 12 ml/min; the perfusate dripped from the transected left atrium and was collected and recirculated. The total volume of recirculating perfusate was 40 ml. Ventilation pressure was continuously monitored with a pressure transducer (PM 131TC, Statham Instruments, Oxnard, CA) and a direct writing oscillograph (Gould, Inc., Cleveland, OH).

The control gas for lung ventilation was 5% CO_2 in air; all ventilation gases contained 5% CO_2 unless otherwise stated. Perfusate was Krebs-Ringer bicarbonate buffer (pH 7.4) containing 10 mM glucose, 5% (wt/vol) fatty acid–free bovine serum albumin (Boehringer-Mannheim Biochemicals, Indianapolis, IN) and pre-equilibrated with the same gas mixture subsequently used for lung ventilation.

Lungs were perfused for an initial 5-min equilibration period. Perfusion was then discontinued by turning off the pump motor while ventilation continued. Each experiment used one of the following for the ventilating gas during ischemia: 0, 5%, 20%, 50%, or 95% O_2 plus 5% CO_2, balance N_2. Premixed gases were obtained from Airco Inc., Philadelphia, PA. After an ischemic period of 15, 30, or 60 min, perfusion was restarted at the same flow rate and continued for 3 min while ventilating with 95% O_2. The brief reperfusion period was utilized to permit evaluation of lung damage by measurement of fluid accumulation.

At the end of 3-min reperfusion, the lungs were rapidly frozen by clamping with aluminum tongs precooled in liquid N_2. A portion of the frozen lung was weighed (wet weight) and then dried in an oven at 60° to constant weight (dry weight). The remainder of lung tissue was immediately homogenized under N_2 in ice-cold saline (1:10, tissue/medium) containing 0.2% butylated hydroxytoluene using a stainless steel micro jar attached to a Waring Blendor (Thomas Scientific, Philadelphia, PA) at maximum speed for 2 min. The homogenate was frozen at $-80°$ under N_2 and was assayed within 3 d. TBARS were assayed in the homogenate after extraction with boiling trichloroacetic acid by the method of Buege and Aust (12) and expressed as picomoles per milligram protein (12). A portion of the lung homogenate was extracted by the method of Bligh and Dyer (13) and used to assay for conjugated dienes as described by Thom (14). Briefly, the organic fraction was removed by evaporation under N_2 and the lipid residue was dissolved in chloroform/methanol (1:6, vol/vol) for measurement of absorbance at 233 nm using a spectrophotometer (model U-3210, Hitachi Instruments Inc., Danbury, CT). Units for conjugated dienes are expressed as millioptical density units per milligram of protein. To measure lung ATP content, a portion of the lung homogenate was extracted with cold ethanolic perchloric acid and assayed enzymatically using hexokinase and glucose-6-phosphate dehydrogenase (15). Protein content of the lung homogenate and extracts was measured using the Coomassie Blue reaction (16).

Results are generally expressed as mean±SE. Data were analyzed statistically by factorial analysis of variance (ANOVA) for the effect of increasing O_2 or by ANOVA followed by Dunnett's test for other comparisons (17). The level of statistical significance was taken as $P < 0.05$.

Results

Lungs evaluated immediately after the equilibration period before production of ischemia showed values for tissue TBARS, ATP, and dry/wet weight (Table I) similar to previously published values for perfused lungs (15, 18). Control lungs which were perfused for an additional 60 min (no ischemia) and ventilated with either 20% or 95% O_2 showed no significant change in tissue TBARS, conjugated dienes, ATP, or dry/wet weight (Table I).

TBARS in lung homogenate increased significantly with ischemia and showed a dependence on time of ischemia and alveolar O_2 concentration (Fig. 1). With ischemia for 60 min,
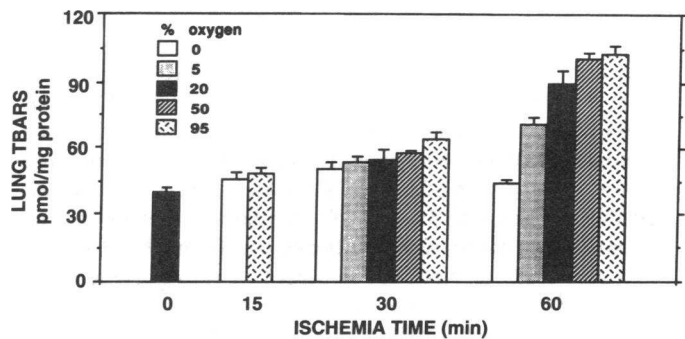

Figure 1. The effect of oxygen content of the ventilating gas on lung TBARS. Isolated rat lungs were ischemic for 15, 30, or 60 min during ventilation with gas of varying oxygen concentrations as indicated. All gas mixtures for ventilation contained in addition 5% CO_2, balance N_2. The zero ischemia time is the same value as in Table I for 0-min perfusion. Results are mean±SE for $n = 4$–8. The increase in TBARS as a function of O_2 was statistically significant ($P < 0.05$) (see text) for 60-min ischemia.

TBARS increased by 162% above the control (no ischemia) lungs during ventilation with 20% oxygen. Ventilation with N_2 during ischemia effectively abolished the increase of lung TBARS, whereas ventilation with 5% O_2 resulted in a tissue TBARS content between the N_2 and 20% O_2 values. There was essentially no change in tissue TBARS between 50% and 95% alveolar O_2, suggesting a plateau of the O_2 effect. The increase of lung TBARS as a function of O_2 concentration was statistically significant ($F = 43.08$; df = 4; $P < 0.05$) by ANOVA using unweighted means analysis for unequal n (17). With 30 min of ischemia, TBARS showed a similar pattern as with 60 min although a lesser change was observed at each O_2 concentration and the O_2 effect was not statistically significant (Fig. 1). A small elevation of TBARS with 15 min of ischemia (studied with N_2 and 95% O_2 only) was also not significantly different than control values (Fig. 1).

We determined whether the 3-min reperfusion period following ischemia was necessary for the generation of lipid peroxidation products. Lungs were ventilated with 20% O_2 during 60-min ischemia and assayed for TBARS without reperfusion. Lung TBARS were 84.0±6.6 pmol/mg protein ($n = 4$), not significantly different from lungs that had been ventilated with 20% O_2 for 60 min and reperfused for 3 min (Fig. 1 and Table II). Therefore, the increase in tissue TBARS occurred during the ischemic period and not during the brief reperfusion.

Conjugated dienes were evaluated as a separate index of tissue lipid peroxidation and gave results essentially parallel to

Table I. Control Studies for Isolated Perfused Rat Lungs

Duration of perfusion	O_2 in ventilating gas	Tissue TBARS	Conjugated dienes	ATP	Dry/wet wt
min	*%**	*pmol/mg protein*	*mOD/mg protein*	*μmol/g dry wt*	
0‡	20	38.4±1.8	4.9±0.3	9.3±0.6	0.180±0.001
60	20	33.6±1.8	4.6±0.07	9.0±0.2	0.181±0.002
60	95	36.0±1.8	5.0±0.48	ND	0.180±0.002

Results are mean±SE for $n = 4$–7. ND, not determined. * Ventilation gas contained in addition 5% CO_2, balance N_2. ‡ Cleared of blood only.

that observed for TBARS (Fig. 2), although no plateau value was observed. For all data, there was a statistically significant correlation between conjugated dienes and TBARS ($r = 0.78$; $n = 58$; $P < 0.001$). With ischemia for 60 min and 20% O_2 ventilation, the tissue conjugated dienes increased 65%. The tissue content of conjugated dienes increased further when lungs were ventilated with 50% or 95% O_2 during ischemia and was significantly less with 5% or 0% O_2 ventilation. The effect of O_2 on conjugated dienes was statistically significant ($F = 16.25$; df $= 4$; $P < 0.05$) by ANOVA as described above. There was a similar O_2 dependence during 30-min ischemia but the elevations were relatively small and were not statistically significant (Fig. 2). With 15-min ischemia, the increase in tissue conjugated dienes was also not statistically significant (Fig. 2).

In order to determine the role of eicosanoid metabolism in the generation of lung lipid peroxidation products, we evaluated the effect of indomethacin on production of TBARS and conjugated dienes. Anesthetized rats were given 20 mg of indomethacin into the dorsal vein of the penis 10 min before removal of lungs which were then perfused at 12 ml/min with medium containing 30 mM indomethacin for 63 min (perfused), or for a 5-min equilibration period before cessation of perfusion (ischemic). Ischemia was continued for 60 min, during which lungs were ventilated with 5% CO_2 in air, followed by reperfusion at 12 ml/min for 3 min. This regimen for indomethacin treatment essentially abolished production of thromboxane B_2 assayed in the lung homogenate and perfusion medium with a radioimmunoassay kit (Amersham Corp., Arlington Heights, IL) (data not shown) consistent with previous results of others with perfused rabbit lungs (19). Ischemia produced a significant increase in TBARS in ischemic lungs even in the presence of indomethacin. Although TBARS after ischemia were 15% less in indomethacin treated lungs compared with control ischemic lungs (no indomethacin) this effect was not statistically significant (Table II). Values for conjugated dienes in the ischemic lung treated with indomethacin (6.8 ± 0.7 mOD/mg protein) indicated a similar effect. Additional experiments ($n = 4$) utilized 100 μM 5-, 8-, 11-, 14-eicosatetraynoic acid (ETYA, BIOMOL Research Laboratories, Inc., Plymouth Meeting, PA), an inhibitor of both lipoxygenase and cyclooxygenase pathways (20), added to the perfusate during the equilibration period. Lungs were ventilated with 5%

Table II. Effect of Eicosanoid Metabolism Inhibitors on Generation of TBARS by the Isolated Rat Lung

	TBARS		
	Control*	Indomethacin	ETYA
	pmol/mg protein		
Perfused	33.6±1.8	29.1±1.7	35.7±2.2
Ischemic	87.9±5.7[‡]	75.0±4.8[‡]	64.8±6.0[‡§]
Mean change	54.3	45.9	29.1

All lungs were ventilated with 20% O_2/5% CO_2/balance N_2. Control lungs were perfused at 12 ml/min for 63 min. Experimental lungs were ischemic for 60 min followed by reperfusion for 3 min. Results are mean±SE for $n = 4$. See text for concentrations of inhibitors.
* Results for perfused from Table I and for ischemic from Fig. 1.
[‡] $P < 0.05$ vs. perfused.
[§] $P < 0.05$ vs. control.

CO_2 in air during 60 min of ischemia. TBARS were significantly increased by ischemia comparing perfused and ischemic ETYA-treated lungs (Table II). However, lung TBARS after 60 min of ischemia in the presence of ETYA was significantly decreased by 46% compared with control (minus ETYA) (Table II). There was no significant effect of either eicosanoid inhibitor on TBARS in normally perfused lungs (Table II). Therefore, total eicosanoid metabolism apparently accounted for nearly half of lung lipid peroxidation products due to ischemia. The greater effect of ETYA compared with indomethacin suggests that the major component of eicosanoid-related lipid peroxidation was due to lipoxygenase activity.

The normal lung has a very large blood flow in relation to tissue mass, far in excess of that required for its metabolic needs. In order to assess the role of perfusion flow rate for oxidative injury, lungs were perfused at 1 ml/min (vs. the standard 12 ml/min) for 60 min and ventilated with 20% O_2. Lung TBARS were 42.3 ± 1.8 pmol/mg protein ($n = 4$) and conjugated dienes were 5.1 ± 0.6 mOD/mg protein, results not significantly different from control values. This indicates that a relatively low level of lung perfusion was sufficient to prevent lipid peroxidation.

In order to evaluate the role of possible pH change during the ischemic period, the ischemic lungs were ventilated with gas containing no CO_2 (alkalosis) or 80% CO_2 (acidosis) in 20% O_2. Equilibrium values for perfusate pH under these conditions were 7.8 and 6.6, respectively, compared with the control value of 7.4. Although TBARS were increased under both acidotic and alkalotic conditions (Table III), the difference from usual pH was not statistically significant. Ventilation with 80% CO_2 during normal perfusion (15 ml/min) also did not affect lung TBARS (Table III compared with Table I).

Lung ATP content was significantly decreased during ventilation with N_2 but was maintained (no significant change from the control value of 9.0 ± 0.2) during ischemia when lungs were ventilated with 5% or 20% O_2 (Table IV). This suggests that availability of metabolizable substrate was not limiting for ATP synthesis during the ischemic period. In order to test further the possibility that lipid peroxidation resulted from substrate depletion in the absence of perfusion flow, lungs were
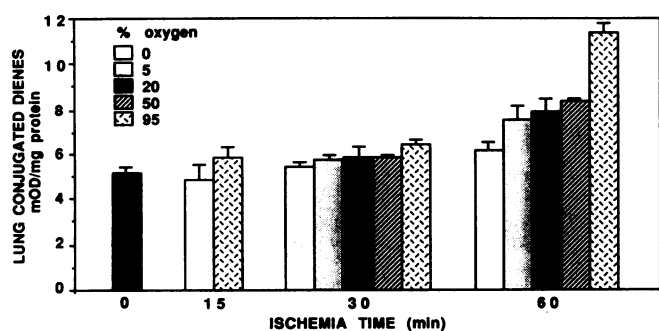

Figure 2. Effect of oxygen content of the ventilating gas on tissue conjugated dienes during lung ischemia for 15, 30, or 60 min. Experimental conditions and presentation of data are indicated in the legend to Fig. 1. The increase in conjugated dienes as a function of O_2 was statistically significant ($P < 0.05$) (see text) for 60-min ischemia.

Table III. Effect of CO_2 in Ventilating Gas on Lung TBARS during Ischemia

CO_2*	Condition	TBARS
%		pmol/mg protein
5	Normal pH	84.0±6.6
0	Alkalosis	94.5±3.3
80	Acidosis	99.3±3.0
80	No ischemia, acidosis	37.2±0.3[‡]

TBARS were measured after 60 min of ischemia, without reperfusion.
Values are mean±SE for $n = 4$.
* CO_2 in the ventilating gas plus 20% O_2 with the balance N_2.
‡ $P < 0.05$ vs. all other conditions.

perfused with medium containing 20 mM glucose (twice the usual concentration) during the equilibration period and then subjected to 60 min ischemia with 20% O_2 ventilation. In additional experiments, lungs were perfused at 1 ml/min with medium containing zero glucose and ventilated with 20% O_2. Lung TBARS under these two conditions ($n = 4$) were 92.4±8.1 and 38.7±3.6 pmol/mg protein, respectively, which is not significantly different from values with the corresponding standard (10 mM) glucose concentration. Therefore, glucose depletion during ischemia did not appear to play a role in promoting lung lipid peroxidation.

There was no evidence of alveolar edema in any of these experiments by gross inspection of lungs. Ventilation pressure for the lungs at constant tidal volume remained constant under all experimental conditions of variable ischemia duration and oxygenation and did not change significantly during the 3-min reperfusion period (data not shown). Tissue dry/wet weight measured at the end of the reperfusion period was unchanged during 60 min of ischemia when lungs were ventilated with N_2 (Table V). However, lungs ventilated with increased concentrations of O_2 showed an oxygen- and time-dependent decrease in dry/wet weight indicating an accumulation of fluid (Table V), presumably occurring during the 3-min reperfusion period. The effect of O_2 was statistically significant ($F = 8.93$; df $= 4$; $P < 0.05$) for the 60-min ischemia values. There was a statistically significant correlation of lung dry/wet weight with

Table IV. Effect of O_2 during Ischemia on Lung ATP Content

	ATP	
O_2*	30-min ischemia	60-min ischemia
%	μmol/g dry wt	
0	5.2±0.1[‡]	4.1±0.06[‡§]
5	8.2±0.2	8.1±0.5
20	8.4±0.1	8.2±0.1

Values are mean±SE for $n = 4$ for each condition.
ATP was measured after 3 min of reperfusion.
* O_2 content of the ventilating gas plus 5% CO_2, balance N_2.
‡ $P < 0.05$ vs. other % O_2 values at the same time point.
§ $P < 0.05$ vs. corresponding 30-min value.

Table V. Effect of O_2 during Ischemia on Lung Dry/Wet Weight

	Dry weight × 100/wet weight		
	Ischemia time *min*		
O_2*	15	30	60[‡]
%	g × 100/g		
0	18.5±0.6	18.0±0.4	18.2±0.3
5	ND	17.8±0.4	17.2±0.4
20	ND	17.6±0.7	15.4±0.1
50	ND	17.4±0.2	15.2±0.1
95	19.0±0.5	17.2±0.4	14.8±0.2

Values are mean±SE for $n = 4$–8 for each condition.
Values were obtained at the end of 3 min of reperfusion.
ND, not determined.
* O_2 content of the ventilating gas plus 5% CO_2, balance N_2.
‡ $P < 0.05$ for the effect of O_2.

TBARS ($r = -0.76$; $n = 58$; $P < 0.001$) and with conjugated dienes ($r = -0.62$; $n = 58$; $P < 0.001$).

Discussion

These studies used the isolated rat lung model to investigate oxidative injury during lung ischemia. Ischemia was produced by discontinuing perfusion to the lungs while ventilation continued in order to maintain and vary tissue oxygenation. Consequently, we were able to evaluate the role of O_2 tension during lung ischemia in the production of lung injury, without invoking potential systemic influences as might occur if the experiment were performed in situ. These isolated lungs were perfused with artificial media so that blood components and cellular elements did not contribute to the tissue changes seen with ischemia.

Lipid peroxidation was evaluated by the measurement of lung tissue TBARS and conjugated dienes, components that are generated at opposite ends of the lipid peroxidation cascade. Thus, conjugated dienes represent the initial phase of oxidative attack on a complex lipid whereas TBARS represent in large part the final stage related to the decomposition of peroxidized lipids (12). The close correspondence of two different parameters in response to experimental manipulation increases the level of confidence in their use as indices of tissue lipid peroxidation (20). The increase in lipid peroxidation products could represent either the generation of eicosanoids from arachidonic acid or the less specific peroxidation of membrane or other tissue lipids. Based on studies with inhibitors of the cyclooxygenase and lipoxygenase pathways, ~ 40–50% of the increase in lipid peroxidation products in this study was related to activation of eicosanoid metabolism by ischemia, compatible with previous reports of increased eicosanoid production during oxidative injury (19). Conversely, more than half of the lipid peroxidation products presumably arose from attack on tissue lipids.

The results of the present study indicate that peroxidation of lung lipid occurs during a period of lung ischemia and that a reperfusion phase is not necessary for oxidative lung injury.

This result is compatible with a recent study of rat intestine using salicylate as a radical trap which indicated increased production of hydroxyl radicals during ischemia (21). The source of O_2 for radical production in the latter study was not determined but presumably represents residual tissue O_2 and diffusion from the environment. In the present study, peroxidation increased with increasing O_2 in the ventilating gas during ischemia and was significantly inhibited when lungs were ventilated with N_2. Although there was a slight increase in peroxidation products at 15 min of ischemia and a further increase at 30 min, the change from control was not statistically significant until 60 min of ischemia. We interpret these results to indicate that lipid peroxidation is initiated during lung ischemia and that the presence of O_2 is required for the propagation of the chain reaction resulting in the generation of peroxidized lipid products. These results are compatible with the hypothesis put forth for other organs that reperfusion provides O_2 for the lipid peroxidation that had been initiated during tissue ischemia (1). Elevated O_2 during reperfusion has been shown to result in increased brain injury (22) and increased generation of TBARS in tissue slices of rat liver and kidney (23).

Although this study has not specifically identified the initiating events during lung ischemia that result in lipid peroxidation, we have obtained evidence related to several possibilities. First, perfusate neutrophils were not present and therefore were not required for the ischemic injury, compatible with previous studies of ischemia-reperfusion lung injury (10). Although these experiments could not exclude an effect of marginated neutrophils in lungs, PMN were not observed in electron micrographs from ischemic lungs (results not shown). Further, evaluation of similar perfused lungs for isolation of cells by enzymatic digestion indicates a minimal polymorphonuclear leukocyte population (24), suggesting that marginated neutrophils are probably not involved. The prevention of lipid peroxidation when lungs were perfused at 1 ml/min, or $\sim$ 8% of the control rate of lung perfusion, suggests that a pulmonary perfusate factor inhibited the initiation stage. Possibilities for the perfusate factor include the provision of substrate or the removal of metabolites, such as H^+. We were not able to prevent lipid peroxidation by alkalosis, reproduce it through acidosis produced by ventilation with high CO_2 or to alter the effect by manipulation of perfusate glucose. Thus, the precise initiating factors for lipid peroxidation during total lung ischemia remain to be determined.

The rat lung model in the present studies relied on cessation of perfusion to produce global ischemia while ventilation continued. This model may have relevance for the in situ lung in which there is acute obstruction of blood flow (e.g., pulmonary embolism). As with the present rat model, ventilation should be initially preserved to a lung segment with acutely obstructed perfusion. In the rat model, ventilation with N_2 protected the lungs against lipid peroxidation during lung ischemia and prevented the accumulation of fluid during a brief (3 min) reperfusion period. These results are consistent with our previous physiologic and morphologic observations in intact dogs that ischemic lungs ventilated with N_2 (plus 5% CO_2) did not show alterations of lung function following reperfusion (25). Further, Koyama et al. (8) showed with isolated canine lung lobes that N_2 ventilation during ischemia prevented lobar edema during reperfusion. The results suggest that the absence of O_2 protects lung tissue from ischemic damage and, conversely,

ventilation of lungs with enriched O_2 mixtures during a period of total vascular occlusion may promote tissue oxidative damage. The implications for therapy of pulmonary embolism or other lung vascular insult is to maintain the lowest possible inspired O_2 compatible with oxygenation of the arterial blood.

The present results also have relevance for our understanding of pulmonary oxygen toxicity. The rat, which has been studied extensively for hyperoxic injury, demonstrates changes in lung metabolic function after $\sim$ 24 h of breathing 1 atmospheres absolute O_2 (26, 27) with progressive pulmonary changes culminating in death at about 72 h (28). The present results provide evidence for oxidative injury by O_2 within a relatively short time frame (30–60 min) in the abnormal lung. These data indicate that even normal levels of alveolar oxygen may result in tissue oxidative damage under circumstances of tissue ischemia and raise the possibility that alterations of perfusion during O_2 exposure may increase susceptibility to lipid peroxidation.

Acknowledgments

References

1. McCord, J. M. 1985. Oxygen-derived free radicals in postischemic tissue injury. *N. Engl. J. Med.* 312:159–163.

2. Granger, D. N., J. M. McCord, D. A. Parks, and M. E. Hollwarth. 1986. Xanthine oxidase inhibitors attenuate ischemia-induced vascular permeability changes in the cat intestine. *Gastroenterology.* 90:80–84.

3. Halliwell, B. 1987. Oxidants and human disease: some new concepts. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 1:358–364.

4. Cross, C. E., B. Halliwell, E. T. Borish, W. A. Pryor, B. N. Ames, R. L. Saul, J. M. McCord, and D. Harman. 1987. Oxygen radicals and human disease. *Ann. Intern. Med.* 107:526–545.

5. Barie, P. S., T. S. Hakim, and A. B. Malik. 1981. Effect of pulmonary artery occlusion and reperfusion on extravascular fluid accumulation. *J. Appl. Physiol.* 50:102–106.

6. Bishop, M. J., E. S. Boatman, T. D. Ivey, J. P. Jordan, and F. W. Cheyney. 1986. Reperfusion of ischemic dog lung results in fever, leukopenia and lung edema. *Am. Rev. Respir. Dis.* 134:752–756.

7. Bishop, M. J., E. Y. Chi, and F. W. Cheney, Jr. 1987. Lung reperfusion in dogs causes bilateral lung injury. *J. Appl. Physiol.* 63:942–950.

8. Koyama, I., T. J. K. Toung, M. C. Rogers, G. H. Gurtner, and R. J. Traystman. 1987. O_2 radicals mediate reperfusion lung injury in ischemic O_2-ventilated canine pulmonary lobe. *J. Appl. Physiol.* 63:111–115.

9. Kennedy, T. P., N. V. Rao, C. Hopkins, L. Pennington, E. Tolley, and J. R. Hoidal. 1989. Role of reactive oxygen species in reperfusion injury of the rabbit lung. *J. Clin. Invest.* 83:1326–1335.

10. Deeb, G. M., C. M. Grum, M. J. Lynch, T. P. Guynn, K. P. Gallagher, A. G. Ljungman, S. F. Bolling, and M. L. Morganroth. 1990. Neutrophils are not necessary for induction of ischemia-reperfusion lung injury. *J. Appl. Physiol.* 68:374–381.

11. Fisher, A. B., C. Dodia, and J. Linask. 1980. Perfusate composition and edema formation in isolated rat lungs. *Exp. Lung Res.* 1:13–22.

12. Buege, J. A., and S. D. Aust. 1978. Microsomal lipid peroxidation. *Methods Enzymol.* 52:302–310.

13. Bligh, E. B., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37:911–917.

14. Thom, S. R. 1990. Antagonism of carbon monoxide-mediated brain lipid peroxidation by hyperbaric oxygen. *Toxicol. Appl. Pharmacol.* 105:340–344.

15. Bassett, D. J. P., and A. B. Fisher. 1976. Metabolic response to carbon monoxide by isolated lungs. *Am. J. Physiol.* 230:658–663.

16. Bradford, M. M. 1976. A rapid and sensitive method for quantitation of microgram quantities of proteins utilizing the principle of protein dye binding. *Anal. Biochem.* 72:248–254.

17. Winer, B. J. 1971. Statistical Principles in Experimental Design. 2nd edition. McGraw-Hill Book Co., Inc., New York. 309–335 and 384–388.

18. Petruska, J. M., S. H. Y. Wong, F. W. Sunderman, Jr., and B. T. Mossman. 1990. Detection of lipid peroxidation in lung and in bronchoalveolar lavage cells and fluid. *Free Radical Biol. Med.* 9:51–58.

19. Farrukh, I. S., J. R. Michael, S. R. Peters, A. M. Sciuto, N. F. Adkinson, Jr., H. S. Freeland, A. Paky, E. W. Spannhake, W. R. Summer, and G. H. Gurtner. 1988. The role of cyclooxygenase and lipoxygenase mediators in oxidant-induced lung injury. *Am. Rev. Respir. Dis.* 137:1343–1349.

20. Smith, C. V., and R. E. Anderson. 1987. Methods for determination of lipid peroxidation in biological samples. *Free Radical Biol. Med.* 3:341–344.

21. Udassin, R., I. Ariel, Y. Haskel, N. Kitrossky, and M. Chevion. 1991. Salicylate as an in vivo free radical trap: studies on ischemic insult to the rat intestine. *Free Radical Biol. Med.* 10:1–6.

22. Mickel, H. S., Y. N. Vaishnav, O. Kempski, D. von Lubitz, J. F. Weiss, G. Feurestein. 1987. Breathing 100% oxygen after global brain ischemia results in increased lipid peroxidation and increased mortality. *Stroke.* 18:426–430.

23. Salaris, S. C., and C. F. Babbs. 1989. Effect of oxygen concentration on the formation of malondialdehyde-like material in a model of tissue ischemia and reoxygenation. *Free Radical Biol. Med.* 7:603–609.

24. Fisher, A. B., L. Furia, and H. Berman. 1980. Metabolism of rat granular pneumocytes isolated in primary culture. *J. Appl. Physiol.* 49:743–750.

25. Fisher, A. B., R. W. Hyde, and J. S. Reif. 1972. Insensitivity of the alveolar septum to local hypoxia. *Am. J. Physiol.* 223:770–776.

26. Gacad, G., and D. Massaro. 1973. Hyperoxia: influence on lung mechanics and protein synthesis. *J. Clin. Invest.* 52:559–565.

27. Block, E. R., and A. B. Fisher. 1977. Depression of serotonin clearance by rat lungs during oxygen exposure. *J. Appl. Physiol.* 42:33–38.

28. Clark, J. M., and C. J. Lambertsen. 1971. Pulmonary oxygen toxicity: a review. *Pharmacol. Rev.* 23:37–133.