The Effect of Hyperoxia on the Lungs of Rats Deficient in Essential Fatty Acids

Ryusuke MURAKAMI, Hidefumi OBARA*, Tetsuya MOMOTA, Akiyoshi TANAKA, Hajime NAKAMURA, Katsuya MIKAWA*, and Seizo IWAI*

Morphological alterations in the lungs of rats deficient in either or both of vitamin E and essential fatty acids were investigated after exposure to hyperoxia for 48 h. In rats deficient in both vitamin E and essential fatty acids, there was damage to type-2 alveolar cells observed as swollen mitochondria and bleb formation in the cytoplasm. None of these changes was found in rats deficient in only one of these substances. Hyperoxia in rats deficient in both substance also caused destruction of the capillary endothelial cells and edema in the interstitium. The lungs of rats deficient in only one of the substances showed some edema in the capillary endothelial cells, but not destruction, and less interstitial edema. These findings suggest that simultaneous deficiency in vitamin E and essential fatty acids facilitates lung damage in rats exposed to hyperoxia. (Key words: oxygen toxicity, essential fatty acids, vitamin E)

(Murakami R, Obara H, Momota T et al.: The effect of hyperoxia on the lungs of rats deficient in essential fatty acids. J Anesth 3: 149–154, 1989)

Exposure to high concentrations of oxygen is a commonly employed therapy for respiratory distress syndrome of premature babies, but this therapy can damage the lung tissue[1–3]. The damage to lungs exposed to a high concentration of oxygen is thought to be related to vitamin E levels and to the fatty acid content in the cell membrane.

Several investigators have demonstrated that vitamin E deficiency in animals enhances the toxic effect of oxygen on the lungs[4–6]. However, the effect of essential fatty acid deficiency, with or without vitamin E deficiency, on oxygen toxicity to the lungs has not been reported. Human newborn infants suffering from respiratory distress syndrome cannot take enough milk orally, so they are given parenteral nutrition that includes glucose, amino acids and fat emulsion. Premature infants given parental nutrition easily develop both vitamin E and essential fatty acid deficiency[7–9]. The purpose of this study was to examine the effect of oxygen toxicity on lung tissue in animals deficient in both vitamin E and essential fatty acids.

Materials and Methods

Wistar male rats, weighing 100–112 gm, were put into four groups. The control group was fed a standard lab chow for 4 months; the group with essential fatty acid deficiency (EFAD) was given a fat-free diet for 4 months; the group with vitamin E deficiency (Vit-ED) was given a diet free of vitamin E for 4 months; the group deficient in both vitamin E and essential fatty acids was given a diet free of both essential fatty acids and vitamin E for 4 months. Diet and water

Department of Pediatrics and Anesthesiology, Kobe University School of Medicine, Kobe, 650 Japan*

Address reprint requests to Dr. Obara: Department of Anesthesiology, Kobe University School of Medicine, Kusunokicho 7, Chuoku, Kobe, 650 Japan

Table 1. Body weight, plasma vitamin E, and fatty acid composition of plasma phosphatidylcholine in rats

	No.	Oxygen exposure	Body weight (g)	Vitamin E ($\mu g/m\ell$)	Fatty acids	
					$\frac{C_{20:3\omega g}}{C_{20:4}}$	$\frac{\text{Total-EFA}}{\text{Total-non-EFA}}$
EFA deficiency	3	−	320 ± 8.2	10.1 ± 0.76	1.35, 1.00, 0.85	0.16, 0.14, 0.20
	3	+	312 ± 13.1	13.9 ± 1.78	0.84, 0.99, 0.90	0.19, 0.16, 0.18
EFA and vitamin E deficiency	3	−	316 ± 7.9	2.15 ± 0.80	0.78, 1.23, 1.33	0.14, 0.20, 0.20
	3	+	315 ± 14.9	1.39 ± 0.38	0.91, 1.85, 1.42	0.23, 0.18, 0.18
Vitamin E deficiency	3	−	315 ± 4.1	2.66 ± 0.11	ND, ND, ND	0.36, 0.38, 0.36
	3	+	323 ± 10.3	1.07 ± 0.14	ND, ND, ND	0.36, 0.35, 0.36
Control	3	−	410 ± 7.5	10.35 ± 0.4	ND, ND, ND	0.46, 0.44, 0.51
	3	+	415 ± 18.7	10.11 ± 1.19	ND, ND, ND	0.47, 0.47, 0.49

EFA (essential acids) = $C_{18:2} + C_{18:3} + C_{20:4}$
non-EFA (non-essential fatty acids) = $C_{14} + C_{16} + C_{16:1} + C_{18} + C_{18:1} + C_{20:3\omega g}$
ND: Not detected

were given ad libitum. All test diets and lab chows were purchased from Oriental Yeast Co. (Osaka, Japan).

After 4 months, half of the animals in each group were sacrificed without exposure to a high concentration of oxygen and the rest were sacrificed after exposure to more than 96% oxygen for 48 hours. Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital. The trachea was then exposed and cannulated with a blunt needle. The lungs were fixed via intratracheal instillation of buffered 4% glutaraldehyde at an inflation pressure of 20 cmH_2O. Thereafter, the trachea was ligated, and the lungs and heart were removed en bloc. After fixation in the inflated state, each lobe from the lungs was minced into 2–3 mm^3 pieces, rinsed in a sucrose solution, postifixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Epon. Thin sections were cut with glass knives and stained with uranyl acetate and lead citrate. The tissue was examined with a Hitachi HS-9 electron microscope (Hitachi, Japan) at 75 KV.

Just before the rats were killed, blood was sampled by cervical vein puncture and assayed for both plasma vitamin E and the fatty acid composition of plasma phosphatidylcholine. Plasma vitamin E was assayed using the method of Abe et al.[10] The fatty acids of plasma phosphatidylcholine were analyzed using the method of Paulsrud et al.[11] Holman et al. noted that the ratio of endogenous trienoic acid to essential tetraenoic acie ($C_{20:3w9}/C_{20:4}$) can be used to assess the essential fatty acid status[12]; a ratio of more than 0.4, indicates a deficiency of essential fatty acids.

Results

Table 1 shows the body weight, plasma vitamin E levels, and the fatty acid composition of plasma phosphatidylcholine. In rats kept on a fat-free diet for 4 months, the ratio of $C_{20:3w9}$ to $C_{20:4}$ was more than 0.4, indicating a deficiency in essential fatty acids. Mitochondria in type-2 alveolar cells were more swollen and rounded in rats deficient in vitamin E or in essential fatty acids (fig. 1) than in control rats (fig. 2). In the type-2 alveolar cells in rats deficient in both vitamin E and essential fatty acids, the mitochondria were swollen more than in rats deficient in only one of these substances, and part of the cytoplasm projected into the alveolus, forming blebs. The blebs were broken down in places and intracellular material had entered into the alveolar spaces (fig. 3).

In the control rat lungs exposed to hyperoxia, the type-2 alveolar cells appeared

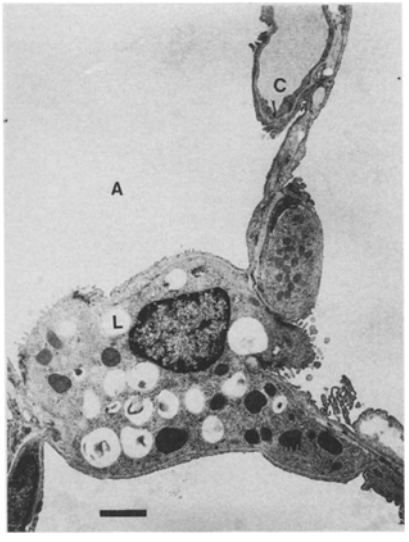

Fig. 1. Type-2 alveolar cell from a rat deficient in essential fatty acids without exposure to hyperoxia. Slight swelling of the mitochondria is seen. ×6000. Bar = 2μ. A, alveolus; C, capillary; L, lamellar body; M, mitochondria

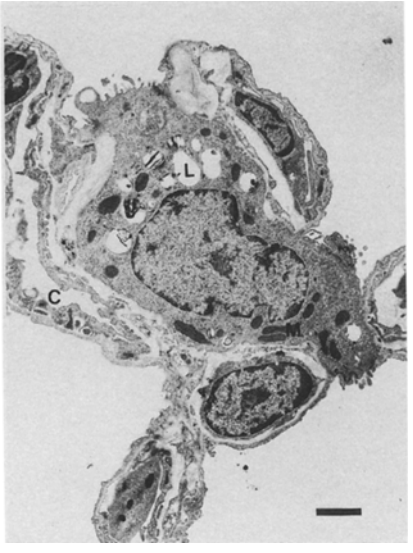

Fig. 2. Type-2 alveolar cell from control rat. ×6000. Bar = 2μ. C, capillary; L, lamellar body; M, mitochondria

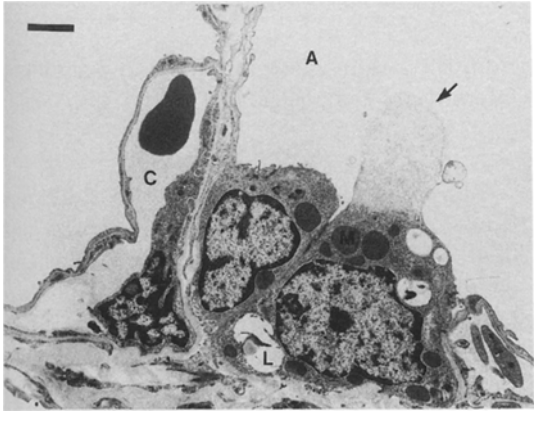

Fig. 3. Type-2 alveolar cell in rat deficient in both vitamin E and essential fatty acids without exposure to hyperoxia. Bleb formation (arrow) and swelling of mitochondria are seen. ×6000. Bar = 2μ. A, alveolus; C, capillary; L, lamellar body

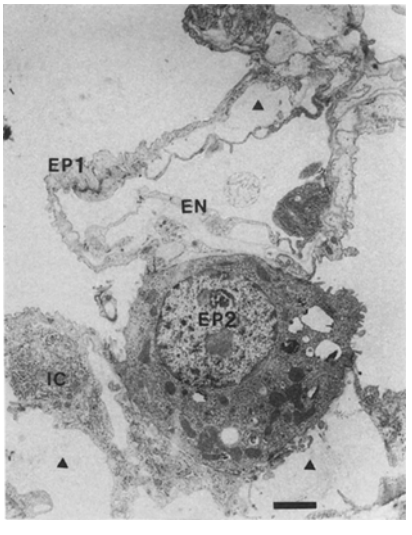

Fig. 4. Interstitial edema in a rat deficient in essential fatty acids after exposure to hyperoxia. ×6000. Bar = 2μ. IC, interstitial cell; EN, capillary endothelium; EP1, alveolar epithelium; EP2, type-2 alveolar cell; ▲, edema

relatively normal. There were focal areas of swollen capillary endothelium, but necrosis and disruption of the capillary endothelium were not observed.

After exposure to high concentrations of oxygen, the lungs in rats deficient in either essential fatty acids or vitamin E had swollen capillary endothelial cells and also interstitial edema compared with the control lung (figs. 4, 5).

In the lungs of rats exposed to hyperoxia and deficient in both essential fatty acids and vitamin E, in addition to the type-2 cell

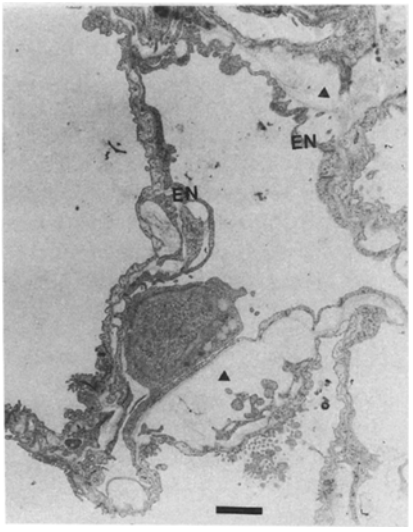

Fig. 5. Interstitial edema, swelling of alveolar epithelium and capillary endothelium are seen in a rat deficient in vitamin E after exposure to hyperoxia. ×6000. Bar = 2μ. EN, capillary endothelium; ▲, interstitial edema

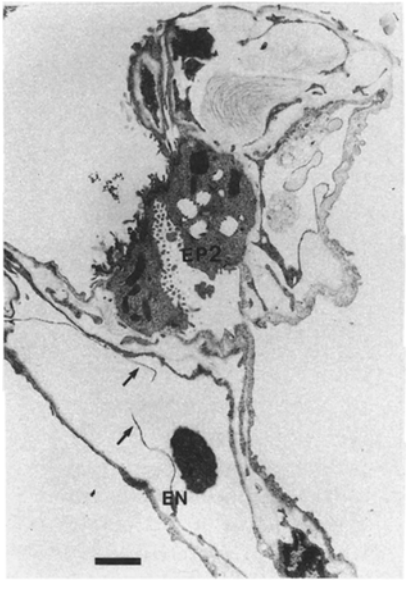

Fig. 7. Lung of rat deficient in vitamin E and essential fatty acids after exposure to hyperoxia. Endothelial detachment from basement membrane (arrow) are seen. ×6000. Bar = 2μ. EN, endothelium; EP1, alveolar epithelium

changes in rats deficient in these 2 substanes, as described above, there were disappearance and detachment of capillary endothelial cells

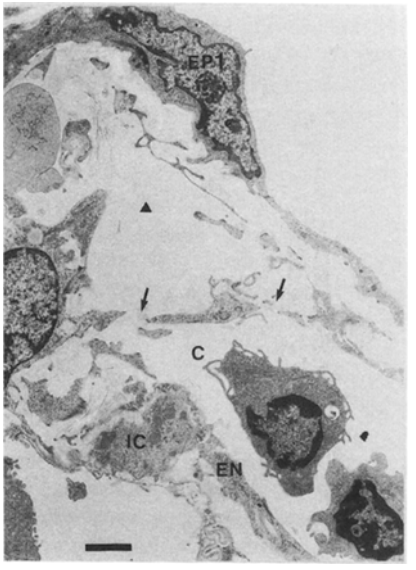

Fig. 6. Lung of rat deficient in vitamin E and essential fatty acids after exposure to hyperoxia. Disruption of capillary endothelium (arrow) and interstitial edema (▲) are seen, ×6000. Bar = 2μ. C, capillary; IC, interstitial cell; EN, endothelium; EP1, alveolar epithelium

from the basement membrane and prominent interstitial edema (figs. 6, 7).

Discussion

Morphological tissue damage to the lungs caused by exposure to high concentrations of oxygen consists of the acute changes including perivascular, peribronchial, interstitial and alveolar edema[13–15]. The toxic effect of oxygen is attributed to the inhibition of sulfhydryl enzymes and lipid peroxidation by the generation of free radicals[16].

Vitamin E plays an important role in protection against the toxic action of free radicals by acting as a scavenger[17,18]. Some investigators showed that vitamin E supplementation to animals exposed to high concentrations of oxygen prevented, or minimized, the biochemical and morphological damage to the lung tissue[19–21]. Also, vitamin E deficiency in animals enhances oxygen toxicity in the lung[1–3]. Newborn infants, particularly premature babies, have low levels of plasma vitamin E, and so may be particularly susceptible to oxygen toxicity.

The hyperoxic exposure time used here was short, but rats deficient in vitamin E showed edema in the capillary endothelial cells and interstitium after 48 h of exposure to oxygen.

Essential fatty acids are also very important nutrients in the neonatal period, because linoleic acid in the plasma and tissue of premature babies is present at low levels[22,23]. The effect of essential fatty acid deficiency on the lung has received little attention. In both this study and that of Edmond et al.[22], changes in mitochondria were observed in the lungs of rat deficient in fatty acids without exposure to a high concentration of oxygen. However, there have been no studies on the effect of hyperoxia on the lungs of animals deficient in essential fatty acids. We found that hyperoxic treatment for 48 h caused additional morphological changes, such as edema of the endothelial cells and interstitium.

The reason why essential fatty acid deficiency enhances oxygen toxicity to lung tissue is not known. Kehrer et al. reported that rats fed on a diet high in saturated fatty acids have decreased polyunsaturated fatty acids in their lung triglycerides, which results in a high mortality rate[22]. He suggested that one reason for the high mortality was that polyunsaturated fatty acids scavenge oxygen radicals and protect against peroxidation of the cell membrane. In our study, the fatty acid composition of triglycerides in the lung was not determined, but the linoleic acid of plasma phosphatidylcholine was greatly decreased in rats deficient in essential fatty acids, which suggests that there was a decrease of linoleic acid in the lung triglycerides. In our study, rats deficient in both vitamin E and essential fatty acids showed a greater susceptibility to hyperoxic damage to the capillary endothelial cells than rats deficient in either one alone. Deficiency of both vitamin E and essential fatty acids in newborn babies suffering from respiratory distress is not uncommon. Our results suggest that a deficiency of both nutrients in these patients may increase hyperoxic lung injury and be implicated in the development of chronic lung disease, such as bronchopulmonary dysplasia.

The authers gratefully acknowledge the review of this paper made by Dr. N. Bery, Department of Biochemistry, Kobe University School of Medicine.

(Received Oct. 26, 1988, accepted for publication Apr. 20, 1989)

References

1. Obara H, Sekimoto M, Iwai S: Alterations to the bronchial and brochiolar surfaces of adult mice after exposure to high concentrations of oxygen. Thorax 34:479–485, 1979
2. Obara H, Pappas CT, Northway WH, Bensch KG: Comparison of the effect of two and six week exposure to 80% and 100% oxygen on the newborn mouse: A quantitative SEM and TEM correlative study. Int Radiation Oncology Biol Phys 11:285–298, 1985
3. Pappas CT, Obara H, Bensch KG, Northway WH: Effect of prolonged exposure to 80% oxygen on the lung of the newborn mouse. Lab Invest 48:735–748, 1983
4. Poland RL: Effect of Vitamin E deficiency in pulmonary oxygen toxicity. Pediat Res 11:577A, 1977
5. Kann HE: Oxygen toxicity and vitamin E. Aerosp Med 35:840–844, 1964
6. Taylor DW: The effect of vitamin E deficiency on oxygen oxicity in the rat. J Physiol 121:49–53, 1953
7. Galdwell MD, Jonsson HT, Otherson HB: Essential fatty acid deficiency in an infant receiving prolonged parenteral alimentation. J Pediatr Surg 81:894–898, 1972
8. White HB, Tuner MD, Tuner AG, Miller RG: Blood lipid alterations in the infants receiving intravenous fat free alimentation. J Pediatr Surg 83:305–313, 1973
9. Gutcher GR, Raynor WJ, Farrell PM: An evaluation of vitamin E status in premature infants. AM J Clin Nutr 40:1078–1089, 1984
10. Abe K, Kusube K, Mukasa K, Ichiguro Y, Sato T, Ichikawa S, Hoshida H: Determination of vitamin A, E and K and ubiquinones in plasma by high speed liquid chromatography. Jap Soci Anal Chem 33:309–314, 1984
11. Paulsrud JK, Pensler L, Whitten CF, Stewart S, Holmen RT: Essential fatty acid deficiency in infants induced by fat-free intra-

venous feeding. Am J Clin Nutr 25:897–904, 1972
12. Holman RT, Caster WO, Wiese HF: The essential fatty acid requirement of infants and the assessment of their dietary intake of linoleate by serum fatty acid analysis. Am J Clin Nutr 14:70–75, 1964
13. Kisler JD, Caldwell PRS, Weibel ER: Development of fine structural damage to alveolar and capillary lining cells in oxygen-poisoned rat lungs. J Cell Biol 32:605–628, 1967
14. Harrison GA: Ultrastructural changes in rat lung during long-term exposure to oxygen. Exp Med Surg 29:96–100, 1971
15. Kapanci Y, Weibel ER, Kaplan HP: Pathogenesis and reversibility of the pulmonary lesions of oxygen toxicity in monkeys. II. Ultrastructural and morphometric studies. Lab Invest 20:101–118, 1969
16. Ludwin SK, Northway WH, Bensch KG: Oxygen toxicity in the newborn: necrotizing bronchiolitis in mice exposed to 100 per cent oxygen. Lab Invest 31:425–435, 1974
17. Wender DF, Thulin GE, Walker SGJ, Wardhaw JB: Vitamin E affects lung biochemical and morphologic response to hyperoxia in the newborn rabbit. Pediatr Res 15:262–268, 1981
18. Tappel AI: Vitamin E as the biological lipid antioxidant. Vitam Horm 20:493–497, 1962
19. Ward JA, Roberts RJ: Vitamin E inhibition of the effects of hyperoxia on the pulmonary surfactant system of the newborn rabbits. Pediatr Res 18:329–334, 1984
20. Bucher JR, Robert RJ: Effects of α-tochopherol treatment on newborn rat lung development and injury in hyperoxia. Pediatr Pharmacol 2:1–9, 1982
21. Friedman Z, Danon Z, Stahlman MT, Oates JA: Rapid onset of essential fatty acid deficiency in the newborn. Pediatrics 58:640–649, 1976
22. Edmonds RH, Beeler DA, Treble DH, Kyriakids EG, Burnell J, Balint JA: Morphological alterations in type-II alveolar cells in essential fatty acid deficiency. Exper Mol Path 23:276–283, 1975
23. Kehrer JP, Autor AP: The effect of dietary fatty acid on the composition of adult rat lung lipid. Relationship to oxygen toxicity. Toxicol Appl Pharmacol 44:423–430, 1978