

An Protective Mechanism against Hyperoxia induced Lipid Peroxidation in the Lung of Vitamin E-deficient Rats

Kimihiko SATO

*The Department of Clinical Pulmonary Physiology, Chest Disease Research Insjitute,
Kyoto University, Kyoto, Japan*

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Lipid peroxides, which produced by oxidation of polyunsaturated fatty acid are highly toxic and damaging to biological system.¹⁾ Lung damage from lipid peroxidation has been well documented in Oxygen toxicity,^{2,3)} Ozone stress,^{4,5,6)} and Paraquat lung^{7,8)} and recent data are suggesting that active oxygen is important etiologic factor inducing ARDS. On the other hand, several protective mechanism against lipid peroxidation are present in biological system. Anti-oxidant enzymes, superoxide dismutase (SOD), catalase, glutathione peroxidase (GSHpx), and α -tocopherol which is main component of Vitamin E are well known as radical scavenger. Although the basic mechanism of protection is gradually resolved, little is understood about how these serve in modulating lung lipid peroxidation. We now described the alterations of the level of TBA-reactants (primarily malonaldehyde, produced by lipid peroxidation), SOD, and GSHpx activities in the lung of Vitamin E-deficient rats exposed to hyperoxia, and discussed the contribution of both enzymes and Vitamin E to detoxification of lung lipid peroxidation.

METHODS

Animals and diets

Male weanling HLA-Wister rats weighing 40 to 50 g were divided into 4 groups: (1) Vitamin E-control diet, non exposure to hyperoxia (control), (2) Vitamin E-control diet, exposure to hyperoxia, (3) Vitamin E-deficient diet, non exposure to hyperoxia, and (4) Vitamin E-deficient diet, exposure to hyperoxia. The composition of the diet deficient in Vitamin E is shown Table I. Vitamin E-control diet contains Vitamin E as dl- α -tocopherol acetate 2 mg/kg diet. Rats were provided these diets for 3 months, and water ad libitum.

Oxygen exposure

Oxygen exposure was done for 24 hours in glass chamber (60 $\times$ 40 $\times$ 30 cm), in which 6 L/min O₂ was provided via two conducting tubes. O₂ and CO₂ concentrations were monitored with Medical Gas Analyser (Perkin Elmer 1100) and maintained $96 \pm 0.4\%$, $4 \pm 0.5\%$, respectively. On the survival experiment, continuous exposure was done for 5 days in the same manner. Tissue preparation and biochemical analysis.

Animals were sacrificed by peritoneal injection of overdose sodiumpentobarbital (150 mg/rat).

Table 1. Composition of basal diet used in this study

Ingredient % of Diet.				
Cornstarch	36%	Vitamin mix		
Vitamin-free casein	25%	A 1,000 IU	D ₃ 200 IU	
Alpha-corn starch	10%	B ₁ 2.4 mg	B ₂ 8.0 mg	
Paper powder	8%	B ₆ 1.6 mg	B ₁₂ 0.001 mg	
Granular sugar	5%	C 60.0 mg	K ₃ 10.4 mg	
Mineral mix	6%	Biotin		0.04 mg
K 420 mg	Fe 27.0 mg	Folic acid		0.4 mg
P 990 mg	Zn 5.1 mg	Ca-pantothenate		10.0 mg
Ca 560 mg	Mn 2.2 mg	P-aminobenzoic acid		10.0 mg
Nd 250 mg	Cu 0.57 mg	Niacin		12.0 mg
Mg 74.9 mg	I 0.46 mg	Inocitol		12.0 mg
Stripped corn oil	8%	Choline-Cl		400.0 mg

Vitamin E-deficient diet group were fed the basal diet alone, and basal diet supplemented with 2 mg dl- α -tocopheryl acetate per kg diet as Vitamin E-control diet.

The thorax was opened and the lung were perfused in situ with ice-cold isotonic saline via the right ventricle to remove as much as intravascular blood possible. Excised lungs were trimmed of extraparenchymal hilar portion, weighed, minced, and homogenized in approximately 10 volumes of ice-cold buffered saline (PBS) with waring blender. This whole-lung homogenate was then centrifuged at $300 \times g$ for 10 minutes. An aliquot of the supernatant was used for determination of TBA-reactants and Vitamin E. The remaining supernatant was then centrifuged at $18,000 \times g$ for 60 minutes. The resulted supernatant was used for measurements of SOD and GSHpx. Vitamin E was determined by high speed liquid chromatographic method of Abe et al.⁹⁾ TBA-reactants were eluted to n-butanol layer by the method of Yagi¹⁰⁾ and measured at an excitation wave length of 515 nm, an emission wave length of 565 nm with Hitachi Spectrophoto Fluorometer 650-10S. The activities of SOD were assayed by its ability to inhibit autoxidation of xanthine oxidase at pH 10.2 by the method of Beauchamp and Fridovich.¹¹⁾ The activities of GSHpx were measured by the method of Paglia and Valentine¹²⁾ and reaction rate was measured at 340 nm, and expressed as the oxidation of 1 μ mol NADPH/minute/protein at 25°C. The protein concentrations were determined by the method of Lowry.

All measurements were performed in duplicate, and analyzed by the Student's t test. Differences were considered significant for $p < 0.05$.

RESULTS

The concentrations of Vitamin E in plasma and lung tissue.

As shown Table 2, the mean plasma and lung Vitamin E levels in rats fed Vitamin E-control diet were $6.39 \pm 0.77 \mu\text{g/ml}$, $7.78 \pm 5.09 \text{ ng/g}$ wet weight, respectively. Rats treated with Vitamin E-deficient diet showed a significant decreases of Vitamin E concentrations of $2.90 \pm 0.46 \mu\text{g/ml}$ in plasma (approximately one half) and of $0.38 \pm 0.03 \text{ ng/g}$ wet weight in lung (approximately one twentieth).

Table 2. The concentration of Vitamin E in plasma and lung

	in plasma	in lung
Vitamin E-control group	$6.39 \pm 0.77 \mu\text{g/ml}$ ($P < 0.001$)	$7.78 \pm 5.09 \text{ ng/g wet weight}$ ($P < 0.001$)
Vitamin E-deficient group	$2.90 \pm 0.46 \mu\text{g/ml}$	$0.38 \pm 0.03 \text{ ng/g wet weight}$

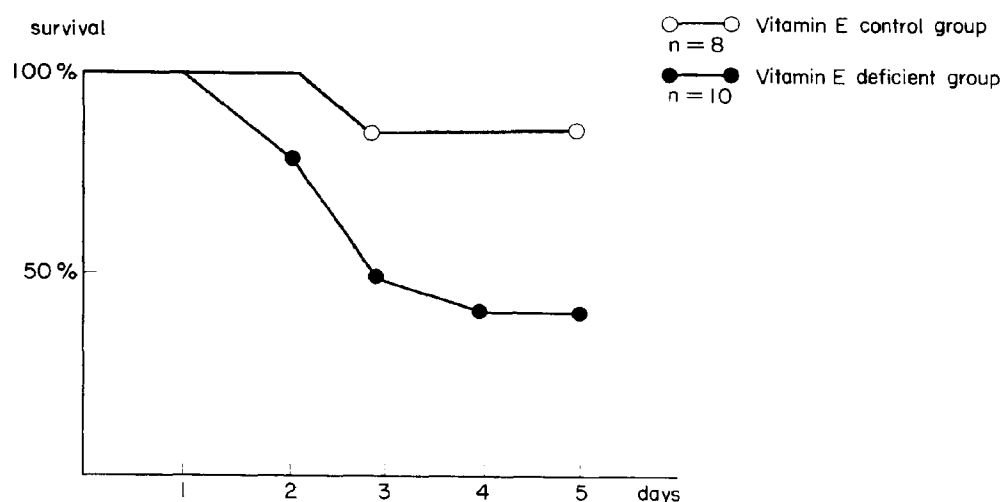
Male weanling rats were provided Vitamin E control or deficient diets (Table 1) for 3 months.

Mortality of rats exposed to hyperoxia

Exposure to 96% oxygen was continued for five days. During oxygen exposure all animals appeared to be inactive and tachypnoeic. Six of ten Vitamin E-deficient rats (60%) died from prominent pulmonary congestion or intraalveolar haemorrhage, while one of eight control rats (12.5%) died within five days. (Fig. 1). The result may explain that oxygen toxicity is intensified in the Vitamin E-deficient rats.

The alterations of TBA-reactants, SOD, and GSHpx.

The results are shown Table 3. A significant increases in TBA-reactants occurred in the lung of rats fed Vitamin E-deficient diet (191.1%), also in the lung of rats exposed to 96% oxygen for 24 hours (120.2%). (data expressed as percent of control values). However, the levels of TBA-reactants in the group of Vitamin E-deficient followed exposure to hyperoxia were as much as in the group of Vitamin E-deficient, non-exposure to hyperoxia. The activities of GSHpx were decreased significantly (32.2%), but no changes of the activities of SOD were detected in the lung of Vitamin E-deficient rats. Exposure to 96% oxygen for 24 hours induced both enzyme activities in the lung of rats. But the degree of increase in the activities of GSHpx (125.6%, $p < 0.02$) was higher than that in the activities of SOD (112.6%, $p < 0.05$) in the Vitamin E-control rats. In contrast to the significant increases in GSHpx activities, no changes in SOD activities were found in the lung of Vitamin E-deficient rats exposed to hyperoxia.



Oxygen concentration was maintained in 96%.

One of 8 control rats and six of 10 Vitamin E-deficient rats died within 5 days.

Figure 1. Effect of hyperoxia on mortality of Vitamin E-deficient rats.

Table 3. Effects of hyperoxia and Vitamin E on TBA-reactants, SOD, and GSHpx of the lung.

Experimental condition	TBA-reactants ¹⁾	SOD ²⁾	GSHpx ³⁾
Vitamin E-control diet and non exposure to hyperoxia (control)	106.5 ± 30.5	1.43 ± 0.17	151.3 ± 13.6
Vitamin E-control diet and exposure to hyperoxia	128.0 ± 16.3 (P < 0.05)	1.61 ± 0.11 (P < 0.05)	190.0 ± 27.1 (P < 0.02)
Vitamin E-deficient diet and non exposure to hyperoxia	203.6 ± 23.2 (P < 0.01)	1.45 ± 0.14	48.7 ± 9.0 (P < 0.001)
Vitamin E-deficient diet and exposure to hyperoxia	196.1 ± 31.6 (P < 0.01)	1.46 ± 0.19	73.8 ± 12.7 (P < 0.01)

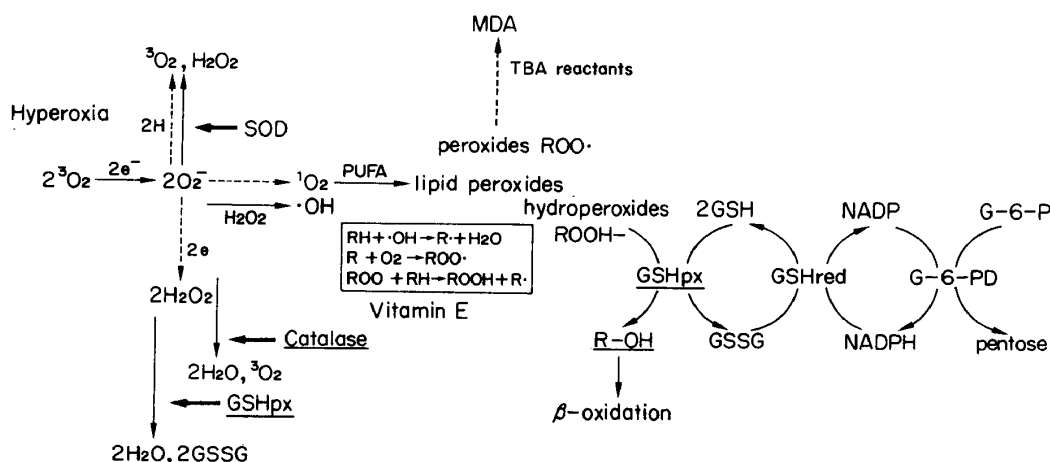
¹⁾: nMol/mg wet weight ²⁾: unit/mg wet weight

³⁾: nMol NADPH oxidized/min/mg protein, 25°C.

DISCUSSION

Prolonged exposure to oxygen of high concentration will cause damage to pulmonary parenchyma. This oxidant injury appears to be mediated by the generation of free radicals, such as the superoxy radical ($\text{O}_2^{\cdot-}$), the perhydroxy radical ($\text{HOO}^{\cdot}$), and the hydroxy radical ($\cdot\text{OH}$), formed in vivo during the univalent reduction of molecular oxygen.^{13,14)} These highly reactive free radicals result in tissue injury through peroxidation of membrane lipids or reaction with sulfhydryl groups to damage enzymes or structural proteins. Among antioxidants SOD catalyzes the reaction $\text{O}_2^{\cdot-} + \text{O}_2^{\cdot-} + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$ and appears to be a primary cellular defence against the superoxy radical.^{15,16)} In the glutathione system, GSHpx inhibits lipid peroxidation by reducing peroxy fatty acids while simultaneously oxidizing glutathione.^{17,18,19)} (Fig. 2).

The results of our experiment which hyperoxia increased in the activities of SOD and GSHpx of the lung are consistent with the reports of previous investigators, and such increases are felt to represent an adaptive response to oxidant injury.^{20,21)} Among chemical antioxidants which may protect the lung from oxidant injury is Vitamin E.²²⁾ Several investigations have demonstrated that Vitamin E-deficiency enhances the toxic effects of oxygen upon the lung,^{23,24)} and Tayler et al.,²⁵⁾ has shown that Vitamin E treatment of Vitamin E-deficient rats significantly

**Figure 2.** Outline of lipid peroxidation by hyperoxia and following degradation of peroxides.

decreases lung damage in animals exposed to oxidant stress. In our observations, increase in mortality of Vitamin E-deficient rats by hyperoxic stress may provide confirmation that Vitamin E has protective mechanism against oxidant injury. Although the protective mechanism of these antioxidants, SOD, GSHpx, and Vitamin E against lipid peroxidation has been revealed gradually, the relations among these antioxidants are not clear. It has been reported that Vitamin E-deficiency did not affect the levels of SOD,²⁶⁾ nor GSH activity.²⁷⁾ Chow²⁸⁾ reported that no alteration of GSH activity was seen in lung while Jensen²⁹⁾ described a decreased GSHpx activity in the liver of rats fed a diet deficient in Vitamin E. Additionally, Chow³⁰⁾ showed that ozone-induced increases in the activity of GSHpx were diminished by the Vitamin E treatment. Wender³¹⁾ also reported that hyperoxia-mediated increases in the pulmonary antioxidant enzymes were diminished by Vitamin E administration. Thus, little is known about how these antioxidant enzymes of lung are affected by Vitamin E condition.

In the present study, a significant decrease in the activity of lung tissue GSHpx was observed in the group fed Vitamin E-deficient diet for 3 months, but such change was not seen in SOD. Induction in GSHpx activity by hyperoxia occurred to Vitamin E-deficient rat although total activity was lower than that of control, and the degree of increase was two fold higher compared to that induced in Vitamin E-control rats by hyperoxia. These observations indicate that Vitamin E and GSHpx have mutually dependent roles. Moreover, GSHpx seems to be more sensitive than SOD and to have main roles of the protective system to respond to oxidative stress. On the other hand, Vitamin E and SOD seems to be independent in protection against oxidative injury, and SOD appears to be secondary importance in this respect. Actually, Hoffman³²⁾ reported no induction in the activity of lung SOD occurred in elder rats upon exposure to 95% oxygen. In this study, we suspect that GSHpx is a specific indicator of oxidative damage, and therefore, should be useful as an index for lipid peroxidation of the lung.

SUMMARY

The effects of hyperoxia on the levels of lung TBA-reactants (an index of lipid peroxidation), superoxide dismutase (SOD), and glutathione peroxidase (GSHpx) were studied in Vitamin E-deficient rats. Male weanling rats were maintained on Vitamin E-deficient or control (containing 2 mg α -tocopherol per 1 Kg diet) diets for 3 months. Compared to control rats, the concentrations of Vitamin E in plasma and lung of Vitamin E-deficient group were decreased one half, one twentieth, respectively.

Vitamin E-deficient rats had TBA-reactants of 191.1% ($p < 0.01$), GSHpx activity of 32.2% ($p < 0.001$), and showed no alteration in SOD activity. (data expressed as percent of control values).

Continuous exposure to 96% oxygen increased in the mortality of Vitamin E-deficient rats. Exposure to 96% oxygen for 24 hours elevated TBA-reactants (120%, $p < 0.05$), as well as the activity of SOD (112.6%, $p < 0.05$), and the activity of GSHpx (125.6%, $p < 0.02$) in the lung of control rats. In Vitamin E-deficient rats, hyperoxia did not increase in the levels of TBA-reactants nor the activity of SOD.

On the other hand, induction of GSHpx by hyperoxia was found in the lung of Vitamin

E-deficient rats although the total activity was lower than that of controls. Thus, GSHpx seems to be more sensitive on the oxidative stress than SOD. The data in the present study suggest that Vitamin E and GSHpx have mutually dependent roles of the protective system against lipid peroxidation, and GSHpx have primary importance on the detoxification of peroxidation induced by hyperoxia.

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