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INDEXING WORDS

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SYNOPSIS

The effects of vitamin E on the lung hyperoxic injury were investigated by the response of lung antioxidant enzymes in young weanling rats exposed to 95% oxygen.

The body weight in hyperoxic rats decreased in the period from 48 to 96 hours of oxygen exposure and it was significantly lower than that of rats under room air at 48 hours or more of oxygen exposure. The administration of vitamin E 15 mg/100g of body weight showed no effect on weight gain. The plasma lipoperoxide level elevated in rats exposed to hyperoxia for 7 days, and the increase was inhibited by the administration of vitamin E.

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Lung antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), were significantly induced in rats exposed to hyperoxia for 3 days and 7 days; however, the catalase activity was not affected by oxygen exposure. The elevation of SOD activity in the lung exposed to hyperoxia was inhibited by the administration of vitamin E.

These findings suggest that the initial response in the antioxidant defense system of lungs against hyperoxia is conducted by the antioxidant enzymes, particularly SOD and GSH-Px; and vitamin E plays a potential role in the defense against the exposure to highly concentrated oxygen.

INTRODUCTION

The etiology of bronchopulmonary dysplasia is most likely multifactorial and relates to endotracheal intubation and positive pressure ventilation, lung edema, and particularly, direct contact with oxygen.[2)]

It is generally believed that lung injury by hyperoxia is induced as a result of the formation of highly reactive free radicals. Superoxide anions (O_2^-) react with unsaturated fatty acids of essential cellular components and the generated lipoperoxides disrupt the normal cellular functions.[10, 19)] In addition, by-products of superoxide anion such as hydroxyl radical ($OH^{\cdot}$), peroxyradical ($HOO^{\cdot}$), hydrogen peroxide, and singlet oxygen ($^1O_2$) also seem to be causative factors of the oxygen-induced cellular injury.[12, 13)]

Antioxidant enzymes, that is superoxide dismutase, glutathione peroxidase, and catalase, have been proposed as playing a role in the potential protective antioxidant defense mechanism.[22, 24)]

We report here the effect of vitamin E administration on the antioxidant mechanism protecting against continuous oxygen exposure in the lungs of young weanling rats by assessing the profiles of the response of antioxidant enzymes.

MATERIALS AND METHODS

Animals

Young Wistar rats 26-days old were housed in individual cages

and given free access to ordinary food (manufactured by Oriental Yeast Inc., Japan) and water.

The animals were divided into following 4 groups.
Group I: No vitamin E supplement and breathing room air;
Group II: Given vitamin E supplement and breathing room air;
Group III: No vitamin E supplement and exposed to hyperoxia;
Group IV: Given vitamin E supplement and exposed to hyperoxia.

A hyperoxic environment was created in a specially designed Plexiglas incubator with two separated chambers; a main chamber with special windows that allow to handle the rats from outside, and small chamber for supplying water and feed and for replacing with fresh cage. Exposure to more than 95% O_2 at ambient pressure was maintained with a flow rate to produce two or three changes in atmosphere per hour. The temperature in the chamber was kept at 20C and the humidity was approximately 100%.

α-Tocopherol acetate (manufactured by Eizai Co. Ltd.) in a dose of 15 mg/100 g body weight was given intra-abdominally before and 24, 48, 96 and 144 hours after the start of the experiment.

A preliminary experiment was performed on nine rats in each group and the body weights were measured every day throughout the experiment. All of the rats survived the experimental period. At 168 hours after the start of the experiment, the blood was collected by cardiac puncture to measure α-tocopherol, lipoperoxide, triglyceride and free fatty acids in plasma.

The experiment was designed to study antioxidant enzymes in lungs. Five or six rats from each group were sacrificed by decapitation at 72 and 168 hours after the start of the experiment, respectively.

The lungs were taken out along with the heart and perfused with phosphate buffer saline to completely wash out the blood in the pulmonary vascular bed. The lungs were excised and trimmed of non-pulmonary tissues and then repeatedly washed in cold phyiologic saline, frozen in liquid nitrogen, and subsequently kept at -70C.

Assays

Lung homogenates were prepared in 10x their weight of cold saline with a homogenizer (2,700 rpm, 10 strokes: Ikemoto Rika Kogyo Co., Ltd., Tokyo). Enzyme activities of SOD and CAT were

analyzed using supernatant of the homogenate centrifuged at 15,000 g for 20 minutes, and GSH-Px activity was measured using supernatant of the homogenate centrifuged at 100,000 g for 20 minutes. The enzymatic activies were measured by the NBT reduction method for SOD,[3] by the method of Cohen et al. for CAT,[5] and by the method of Demus-Oole et al. for GSH-Px.[7] Protein was measured by the method of Lowry et al.[14]

α-Tocopherol in plasma and in lung homogenate was analyzed by high pressure liquid chromatography (Hitachi 635S, Hitachi, Tokyo) and determined by fluorescence spectrophotometry (Hitachi 650-10LC fluorescence spectrophotometer, Hitachi, Tokyo) according to the method of Abe et al.,[1] using the internal standard of α-tocopherol (supplied by Eizai Co. Ltd., Tokyo).

Plasma lipoperoxide was measured by the method of Yagi[23] (Lipoperoxide Test, Wako Junyaku, Tokyo), free fatty acids by the copper salt method (NEFA Test, Wako Junyaku, Tokyo), and triglyceride by the method using glycerol kinase and glycerophosphoric acid oxidase (Lipidos 550, Toyobo Co., Ltd., Tokyo).

Statistics were performed using Student's t-test and two-way analysis of variance. Variation about the mean was expressed as standard error.

RESULTS

Body weight

As shown in Fig. 1, the body weights in the groups exposed to oxygen decreased between 48 and 96 hours after the start of the experiment and thereafter, increased slightly; and they were significantly lower than those in the groups breathing room air between 48 and 168 hours after the start of the experiment. In the groups exposed to hyperoxia, there was no significant difference in the body weights in the group treated with vitamin E in comparison to those in the group not treated with vitamin E.

α-Tocopheraol levels in plasma and lung homogenate

In the groups not treated with vitamin E, the plasma level in the group exposed to oxygen for 7 days was significantly lower than that in the group breathing room air ($p<0.01$); however, the levels in lung homogenate were similar in both groups. Otherwise,

when vitamin E was administered to rats, the α-tocopherol levels in plasma and lung homogenate were significantly higher than those in rats not treated with vitamin E; and the plasma level in the group exposed to oxygen was rather higher than that in the group treated in room air ($p<0.05$), however the level in lung homogenate were comparable in both groups.

Plasma lipoperoxide, triglyceride, and free fatty acids levels

In rats exposed to oxygen for 7 days, the plasma lipoperoxide level showed a slight increase in the group not treated with vitamin E; however, when vitamin E was administered, the plasma lipoperoxide level was significantly lower than that in the group not treated with vitamin E ($p<0.05$).

As for the plasma triglyceride levels, there were no significant differences among the four groupos.

When vitamin E was not administered, there was no difference in the plasma free fatty acids level between the group exposed to oxygen and the group treated in room air. However, with the administration of vitamin E, the plasma free fatty acids level in the group exposed to oxygen was significantly higher than that in the group breathing room air ($p<0.05$).

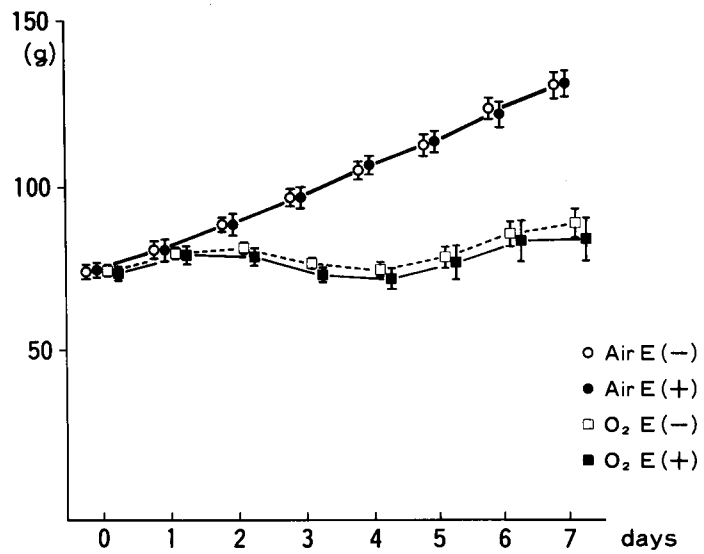

Fig. 1 Body weights during exposure to hyperoxic environment. Mean ± SE.
* $p<0.05$ compared to each room air group.

Antioxidant enzymatic activities in lung homogenate superoxide dismutase

In the groups not treated with vitamin E, the activity of superoxide dismutase in the groups exposed to oxygen for 3 and 7 days were significantly higher than those in the groups breathing room air ($p<0.01$, $p<0.01$ respectively). Otherwise, when vitamin E was administered, the SOD activity in the group exposed to oxygen was similar to that in the group treated with room air; and it was significantly lower than that in the group not treated with vitamin E ($p<0.05$).

Table 1 The effect of vitamin E administration on α-tocopherol level of the plasma and lung homogenate. Mean ± SE.

	Oxygen exposure	Groups			
		not treated with Vit E		treated with Vit E	
		Room air	Hyperoxia	Room air	Hyperoxia
Plasma (mg/dL)	7 days	0.76 ± 0.05 (9)	0.50 ± 0.07 (9)	1.59 ± 0.18 (9)	6.32 ± 3.28 (9)
Lung homogenate (μg/mg protein)	7 days	0.22 ± 0.01 (9)	0.24 ± 0.01 (9)	0.96 ± 0.11 (9)	2.26 ± 0.56 (9)

( ) : number of animals, * : $p<0.05$, ** : $p<0.01$

Table 2 The effect of vitamin E administration on lipoperoxide, triglyceride, and free fatty acids in plasma. Mean ± SE.

	Oxygen exposure	Groups			
		not treated with Vit E		treated with Vit E	
		Room air	Hyperoxia	Room air	Hyperoxia
Lipoperoxide (nmoles/mL)	7 days	4.19 ± 0.43 (9)	4.70 ± 0.43 (9)	3.56 ± 0.33 (9)	2.78 ± 0.31 (9)
Triglyceride (mg/dL)	7 days	92 ± 8 (9)	96 ± 7 (9)	94 ± 8 (9)	108 ± 4 (9)
FFA (μEg/L)	7 days	395 ± 25 (9)	475 ± 63 (9)	469 ± 35 (9)	674 ± 72 (9)

( ) : number of animals, * : $p<0.05$

Table 3 The effect of vitamin E administration on antioxidant enzymatic activities in lung. Mean ± SE.

	Oxygen exposure	Groups			
		not treated with Vit E		treated with Vit E	
		Room air	Hyperoxia	Room air	Hyperoxia
SOD (U/mg protein)	3 days	73.2 ± 6.7 (5)	108.6 ± 6.2 (5) **	77.9 ± 4.1 (5)	102.6 ± 17.2 (5) *
	7 days	64.7 ± 4.9 (6)	84.7 ± 3.3 (6) **	69.4 ± 10.3 (6)	70.2 ± 4.9 (6)
GSH-Px (nmoles NADPH /min/mg protein)	3 days	23.0 ± 2.1 (5)	44.7 ± 5.0 (5) *	23.7 ± 1.9 (5)	40.6 ± 3.5 (5) *
	7 days	18.8 ± 1.3 (6)	41.7 ± 1.6 (6) *	22.9 ± 1.5 (6)	42.2 ± 3.5 (6) *
CAT (U/mg protein)	3 days	11.6 ± 1.8 (5)	13.2 ± 1.6 (5)	12.5 ± 1.2 (5)	13.8 ± 1.1 (5)
	7 days	12.3 ± 1.0 (6)	14.5 ± 0.9 (6)	12.2 ± 1.4 (6)	13.9 ± 0.9 (6)

( ) : number of animals, * : $p<0.05$, ** : $p<0.01$

DISCUSSION

Active oxygen generation is considered one of the causative factors in the mechanism of oxygen-induced injury, and superoxide dismutase has been proposed as playing an important role in the detoxification.[11, 19]

Crapo et al.[6] reported that SOD activity in lung tissue rose by 50% in rats exposed to 85% oxygen for 7 days, and also, Stevens et al.[20] argued that neonatal rats have a higher resistance to oxygen than adult rats because their SOD level begins to rise earlier than that of adult rats after being exposed to oxygen. Furthermore, the glutathione system functions as an additional antioxidant defense mechanism to protect the lung from the injury by lipoperoxides.[16] Yam et al.[24] reported that the increases in the lung complement of SOD, GSH-Px, glutathione reductase, and glutathione in neonatal rats during oxygen challenge may provide the mechanisms for their increased tolerance of hyperoxia-induced lung injury as compared to the adults. Our study also demonstrated that both SOD and GSH-Px activities in lung tissue rose in young rats exposed to 95% oxygen for 3 days and 7 days, and that CAT activity showed no elevation. These results agree with the study

of Bucher et al.[4] and thus, SOD and glutathione system seems to play a more important role in the mechanism for protecting against oxygen-induced lung injuries.

In our experiment, rats exposed to oxygen became immobile from 3 days of oxygen exposure, showing tachypnea and alinasal respiration, and from 6 days of oxygen exposure, the guantity of ingestion began to increase again and weight gain was observed as well. Their adaptation to a hyperoxic environment appeared to be closely associated with the rise of SOD and GSH-Px levels in the lung tissue.

In 1978, Ehrenkranz et al.[8] reported that the incidence of BPD decreased notably in immature infants who were given vitamin E immediately after their birth. However, they reported later that when the inner pressure in the respiratory tract was lowered during mechanical ventilation in the double blind study performed in the following year, a significant difference was no longer recognized between the vitamin E treated group and the non-treated group, because the incidence of BPD decreased on the whole.[9] The prophylactic effects of vitamin E on developing bronchopulmonary dysplasia have not been established yet in clinical trials,[18] despite the fact it was reported that vitamin E deficient rats could be protected against oxygen-induced injuries by administrating vitamin E.[17] As for the antioxidant mechanism of vitamin E, the following two theories are now advocated: (1) peroxidation of unsaturated fatty acids is prevented by direct scavenging for active oxygen; and (2) the side chain of vitamin E makes it difficult for fatty acids to get peroxidized.[15, 21] Our experiment revealed that an oxygen-induced elevation of the plasma lipoperoxide was inhibited in rats treated with vitamin E. Warshaw et al.[22] demonstrated that vitamin E administration to rabbit pups treated with hyperoxia for 72 hours induced the pulmonary antioxidant enzymes SOD, GSH-Px and glutathione reductase in the lung tissue. The increased activity of antioxidant enzymes is thought to represent an adaptive response to protect tissues from oxidant injury. In our experiment, the activity of SOD in the lung tissue exposed to hyperoxia elevated in the group not treated with vitamin E, however, the elevation of SOD was inhibited in rats treated with vitamin E.

These findings suggest that the initial response in the antioxidant defense system of lungs against hyperoxia is conducted

by the antioxidant enzymes, particaularly SOD and GSH-Px; and vitamin E plays a potential role in the defense against the exposure to highly concentrated oxygen.

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