Effects of Oxygen Inhalation on the Antioxidant Capacity of Lungs, Livers, and Brains in Normal and Vitamin E-Deficient Rats at Various Ages

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Summary The effects of oxygen inhalation for 48 h on the antioxidant capacity of lungs, livers, and brains in normal and vitamin E-deficient rats at various ages were examined. The activity levels of catalase, glutathione peroxidase, and superoxide dismutase, and the level of vitamin E in tissue homogenates were assayed as the indices of antioxidant capacity. Oxygen inhalation mostly decreased antioxidant enzyme activity in lungs. In particular, the catalase activity was much decreased. The glutathione peroxidase activity tended to be decreased. The superoxide dismutase activity was decreased in 32-month-old rats. Vitamin E deficiency did not augment oxidative damage due to oxygen inhalation. There appears to be no age effect on the oxygen-induced decrease in the antioxidant enzyme activities of lungs, except the superoxide dismutase activity in very old rats. Oxygen inhalation had some effects on the antioxidant capacity of livers and brains. For example, oxygen inhalation decreased the vitamin E concentration of livers in 32-month-old, normal rats. These results suggest that the antioxidant capacity of lungs is directly damaged by oxygen inhalation and that the antioxidant capacity of livers and brains is indirectly affected through lung damage. Antioxidant capacity may be maintained without large variation during young and middle ages, but its redundancy for emergency use may be diminished in old age.

Key Words aging, oxygen, antioxidant capacity, catalase, glutathione peroxidase, superoxide dismutase, vitamin E, lung, liver, brain

In exchange for making use of molecular oxygen by respiration, aerobic organisms are always exposed to oxidative stress due to molecular oxygen and related reactive species, and hence they have been provided with antioxidant defense to avert damage due to oxidative stress (1). Antioxidant defense is composed of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, and biological antioxidants such as vitamin E. It has been

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suggested that deteriorative changes in antioxidant defense cause the accumulation of oxidative damage, which is relevant to the aging process (2).

Many reports have been published on age-related changes in the activity levels of antioxidant enzymes (2, 3). However, these previously reported results are inconsistent. Thus, it seems unlikely that the antioxidant capacity is deteriorated in a simple manner with aging, although the reason is unclear why this inconsistency occurs. In addition, there is the possibility that during aging, the antioxidant capacity may remain good enough to prevent oxidative stress under ordinary conditions but its redundancy for emergency use may be diminished. If this is the case, old animals may be more vulnerable under unusually heavy oxidative stress, such as under a high concentration of oxygen, than young animals.

We attempted to examine the effects of oxygen inhalation on the antioxidant capacity of lungs, livers, and brains in normal and vitamin E-deficient rats at various ages to ascertain whether or not the redundancy of antioxidant capacity is diminished in old rats. Our results suggest that the antioxidant capacity may be maintained without large variation during young and middle ages but its redundancy appears to be diminished in old age.

MATERIALS AND METHODS

Animals and preparation of tissue homogenates. Female Fischer 344 rats were obtained at 4 weeks of age from Charles River Japan Inc. (Atsugi, Japan) and maintained in the aged animal supply facility of our institute. For the induction of vitamin E deficiency, rats were fed for 9 weeks from 6, 12, 24, or 30 months of age on a mixture of a powdered vitamin E-free diet and vitamin E-stripped corn oil, both provided by Eisai Research Laboratories (Tokyo); the powdered diet and corn oil were mixed just before feeding. The control diet was a standard animal chow (CRF-1: Oriental Yeast Co. Ltd., Tokyo, Japan), which was powdered before feeding. After rats were sacrificed, tissues were excised and stored in liquid nitrogen until use. For the preparation of 10% tissue homogenates (wet weight/volume), the frozen tissues were thawed on ice and then homogenized with a Polytron homogenizer (Kinematica GmbH, Lucerne, Switzerland).

Oxygen exposure. For oxygen exposure, rats were placed in stainless steel boxes (29.1 × 19.2 × 12.5 cm), into which oxygen was supplied from an oxygen cylinder at the rate of 1.5 liters/min for 48 h. The concentration of oxygen in the boxes was monitored with a Model G-101-Y oxygen analyzer (Iijima Products Manufacturing Co. Ltd., Gamagouri, Japan) and was confirmed to be higher than 96%. Oxygen for medical use, the concentration of which was more than 99.5%, was obtained from Suzusho Medical Co. Ltd. (Tokyo, Japan). After oxygen exposure, the rats were sacrificed.

Biochemical analysis. As described previously (3), enzyme assay and vitamin E (α-tocopherol) analysis were performed. The chemicals used were obtained from commercial suppliers.
Statistical analysis. Statistical analysis of the data was performed using a Macintosh LC III computer (Apple Computer, Inc., Cupertino, CA, U.S.A.) with STAT-VIEW SE software program (Abacus Concepts, Inc., Berkeley, CA). Data are shown as means with the standard error and were assessed for significance by three-way analysis of variance (ANOVA) and Student’s t-test. p values of <0.05 were considered significant.

RESULTS AND DISCUSSION

The effects of oxygen inhalation on the antioxidant capacity of lungs, livers, and brains in normal and vitamin E-deficient rats at 8, 14, 26, and 32 months of age were examined. When rats were exposed to oxygen for 48 h, the activity levels of catalase, glutathione peroxidase, and superoxide dismutase and the concentration level of vitamin E in tissue homogenates were assayed as the indices of antioxidant capacity (Figs. 1–4). In our previous paper (3), we have already reported the activity levels of these antioxidant enzymes and the concentration level of vitamin E in tissue homogenates from normal and vitamin E-deficient rats at various ages, which were maintained under air; incidentally, in Fig. 1 of Matsuo et al. (3), “Lung” should read “Cerebrum” and “Cerebrum” should read “Lung,” and in Fig. 3, “µmol” should read “nmol.” In this paper, we used these levels as the control levels for oxygen inhalation experiments.

Lung

When rats are maintained under a high concentration of oxygen, the lung is exposed directly to oxygen: namely, it is a primary target organ for oxidative damage due to oxygen inhalation. Thus, the lung is expected to be more heavily damaged during oxygen inhalation than other organs. The effects of oxygen inhalation on the antioxidant enzyme activities of lungs in normal and vitamin E-deficient rats at 8, 14, 26, and 32 months of age are shown in Fig. 1. After oxygen inhalation, the catalase activity was decreased by 33–49%, although its decrease for 14-month-old rats was statistically insignificant. This decrease occurred regardless of age or vitamin E status. The glutathione peroxidase activity also tended to be decreased regardless of age or vitamin E status. The extent of the decrease seems to be greater in normal rats (38–49%) than in vitamin E-deficient rats (17–37%). The superoxide dismutase activity remained unchanged between 8 and 26 months of age and then was much decreased at 32 months of age. The average percent decreases were 38 and 57% for normal and vitamin E-deficient rats, respectively, at 32 months of age.

The effect of oxygen inhalation on the concentration of vitamin E in lungs in normal and vitamin E-deficient rats at 8, 14, 26, and 32 months of age is shown in Fig. 4. Oxygen inhalation had no effect on the vitamin E level of lungs in normal and vitamin E-deficient rats at various ages.

Adult rats are well known to be sensitive to oxygen and almost all die within...
Fig. 1. The effects of oxygen inhalation on the antioxidant enzyme activities of lungs in normal and vitamin E-deficient rats at 8, 14, 26, and 32 months of age. Columns indicate the activities of catalase, glutathione peroxidase, and superoxide dismutase in normal rats unexposed (□) and exposed (■) to oxygen and in vitamin E-deficient rats unexposed (■) and exposed (□) to oxygen. Each column represents a mean value for 3–5 rats. Each small bar on the columns represents the standard error of the estimate of a mean value. Differences between each pair of means indicated by the following paired letters are statistically significant (p < 0.05): for catalase, ab, cd, ef, gh, ij, and kl; for glutathione peroxidase, ab, ac, de, fg, and fh; for superoxide dismutase, ab and cd.
Fig. 2. The effects of oxygen inhalation on the antioxidant enzyme activities of livers in normal and vitamin E-deficient rats at 8, 14, 26, and 32 months of age. Notes for columns and error bars are as indicated in the legend to Fig. 1. Differences between each pair of means indicated by the following paired letters are statistically significant \( (p<0.05) \): for catalase, ah, bc, dh, ef, eg, eh, and hi; for glutathione peroxidase, aj, bc, df, dj, eg, hi, and kl.
Fig. 3. The effects of oxygen inhalation on the antioxidant enzyme activities of cerebrums in normal and vitamin E-deficient rats at 8, 14, 26, and 32 months of age. Notes for columns and error bars are as indicated in the legend to Fig. 1. Differences between each pair of means indicated by the following paired letters are statistically significant ($p < 0.05$): for catalase, ab and cd.
Fig. 4. The effects of oxygen inhalation on the concentrations of vitamin E in lungs, livers, and cerebrums in normal and vitamin E-deficient rats at 8, 14, 26, and 32 months of age. Notes for columns and error bars are as indicated in the legend to Fig. 1. Differences between each pair of means indicated by the following paired letters are statistically significant ($p < 0.05$): for lungs, ac, ai, bd, dk, eg, fh, and ij; for livers, ac, am, bd, cj, cm, eh, el, ep, fg, fm, ik, im, jl, mn, mo, and np; for cerebrums, ac, bd, eg, fh, ik, ij, and jl.
several days of oxygen exposure (4). On the other hand, neonatal rats are tolerant to oxygen (5) and adult rats become tolerant to it after 85–90% oxygen pre-exposure (6). This tolerance is thought to result from the augmentation of antioxidant defense in lungs (5, 7, 8). The following observations have been reported. In the lungs of oxygen-exposed neonatal rats, the activities of catalase, glutathione peroxidase, superoxide dismutase, glutathione reductase, and glucose-6-phosphate dehydrogenase were increased (5, 8), and the mRNA levels of catalase, glutathione peroxidase, and superoxide dismutase were increased and the half-lives of these mRNA were prolonged (9, 10). In the lungs of 85–90% oxygen-exposed adult rats, the activities of superoxide dismutase, glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase were increased (7, 11). In the lungs of 96–98% oxygen-exposed adult rats, however, the activities of catalase, glutathione peroxidase, superoxide dismutase, and glutathione reductase were not increased (5, 12).

Our results show that the activities of catalase and glutathione peroxidase in lungs of young and old rats are decreased during oxygen inhalation. The decreases in the activities of catalase and glutathione peroxidase may play an important role in the lethality of oxygen toxicity. After oxygen inhalation, the superoxide dismutase activity of lungs remained unchanged in rats until 28 months of age, but it was decreased in rats at 32 months of age. In addition, we observed that the ratio of unsaturated-fatty-acid to saturated-fatty-acid residues in lung lipids was decreased in normal and vitamin E-deficient rats at 32 months of age (data not shown). The decrease in this ratio is thought to be one of the indices of oxidative damage of lipids. These results suggest that the antioxidant capacity of lungs remains almost unchanged during young and middle ages, but the redundancy of antioxidant capacity may be diminished in old rats.

Liver

The effects of oxygen inhalation on the antioxidant enzyme activities of livers in normal and vitamin E-deficient rats at 8, 14, 26, and 32 months of age are shown in Fig. 2. After oxygen inhalation, the catalase activity tended to be slightly decreased except for that in normal rats at 32 months of age. The glutathione peroxidase activity remained unchanged in rats at various ages except for the increased activity in normal rats at 32 months of age. The superoxide dismutase activity also remained unchanged.

After oxygen inhalation, the vitamin E concentration tended to be decreased in normal rats, and was significantly decreased in normal rats at 32 months of age, while it remained unchanged in vitamin E-deficient rats (Fig. 4). To confirm that oxygen inhalation decreases the antioxidant concentration of livers in normal rats, we measured the induction period and rate of initiator-induced conjugated diene formation in lipid extracts from liver homogenates (Table 1). 2,2'-Azobis(2,4-dimethylvaleronitrile) was used as fat-soluble initiator for radical reactions. As shown in the previous paper (3), the induction period of lipid extracts from livers...
Conjugated diene formation in lipid extracts from the liver homogenates of normal and vitamin E-deficient rats exposed or unexposed to oxygen.

<table>
<thead>
<tr>
<th>Age (month)</th>
<th>Normal vitamin E status</th>
<th>Deficient vitamin E status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Induction period (min/mg lipids) (%)</td>
<td>Rate (nmol conj. diene/min/mg lipids) (%)</td>
</tr>
<tr>
<td></td>
<td>Unexposed</td>
<td>O₂-exposed</td>
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<tr>
<td>8</td>
<td>58.2±2.7</td>
<td>36.9±3.7</td>
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<tr>
<td>14</td>
<td>49.7±2.7</td>
<td>38.6±3.9</td>
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<tr>
<td>26</td>
<td>64.4±8.7</td>
<td>44.5±11.1</td>
</tr>
<tr>
<td>32</td>
<td>73.9±13.3</td>
<td>30.9±3.9</td>
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¹ Conjugated diene formation in lipid extracts in the presence of 2,2'-azobis(2,4-dimethylvaleronitrile) was observed. ² The percentage of each value for oxygen-exposed animals to that for the corresponding unexposed animals was shown. ³ The values shown in the previous paper (3) were used as control values. ⁴ Each difference between the values for oxygen-exposed and unexposed animals is statistically significant (p < 0.05).
is proportional to their vitamin E concentration. Oxygen inhalation shortened the induction period of lipid extracts from normal rats, while it did not shorten that from vitamin E-deficient rats. Presumably, the vitamin E concentration of livers in vitamin E-deficient rats had been much lowered, so that oxygen inhalation could not shorten the induction period. Further, oxygen inhalation increased the rate of initiator-induced conjugated diene formation in lipid extracts from normal and vitamin E-deficient rats. This means that oxygen inhalation decreases the antioxidant concentration of livers and increases their susceptibility to lipid peroxidation after antioxidant exhaustion.

It appears that oxygen inhalation has a considerable effect on the antioxidant concentration of livers. Oxygen may indirectly affect the antioxidant capacity of livers through lung damage; e.g., after being produced in lungs under oxygen exposure, some toxic oxidants, such as 9,10-epoxy-12-octadecenoate (13), might be brought to the liver.

It has been reported that in hepatocytes from rats exposed to oxygen for 48 h, the content of carbonyl groups, an index of protein oxidation, in soluble proteins was much increased and the activities of glutamine synthetase and glucose-6-phosphate dehydrogenase were decreased by 23 and 72%, respectively (14). Such liver damage might result from some oxidants which come from the lungs under oxygen exposure and can not be scavenged by the antioxidant enzymes and vitamin E.

**Brain**

The effects of oxygen inhalation on the antioxidant enzyme activities of cerebrums in normal and vitamin E-deficient rats at 8, 14, 26, and 32 months of age are shown in Fig. 3. After oxygen inhalation, the catalase activity was decreased in normal rats at 26 months of age and in vitamin E-deficient rats at 8 months of age. Further, the activity tended to be decreased in rats other than normal rats at 8 months of age. These may be related to an increase in the hydrogen peroxide formation in the brain in rats under a high concentration of oxygen (15). The glutathione peroxidase and superoxide dismutase activities remained unchanged. The vitamin E level tended to be decreased in old rats (Fig. 4). The ratio of unsaturated-fatty-acid to saturated-fatty-acid residues in cerebrum lipids remained unchanged regardless of age or vitamin E status (data not shown). It has been reported that oxygen inhalation had no effect on conjugated diene formation in lipid extracts from brains (16). Oxygen inhalation may have only a slight effect on the antioxidant capacity of brains.

**Concluding remarks**

These results suggest that the antioxidant capacity of lungs is directly damaged by oxygen inhalation and the antioxidant capacity of livers and brains is indirectly affected through lung damage. Presumably, the oxygen-induced decrease in the catalase and glutathione peroxidase activity levels of lungs may be responsible for lethal damage due to oxygen toxicity. Vitamin E deficiency appears not to augment
oxidative damage due to oxygen inhalation. Antioxidant capacity may be maintained without large variation during young and middle ages, but its redundancy for emergency use may be diminished in old age.

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REFERENCES