PROTECTION WITH CYCLOHEXIMIDE OR EMETINE
AGAINST PULMONARY EDEMA INDUCED BY
OZONE OR NITROGEN DIOXIDE

Ziro Nambu and Eiji Yokoyama

O₃ または NO₂による肺水腫形成に対する
シクロヘキシミドまたはエメチンの前投与の効果

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Pretreatment with cycloheximide or emetine provided significant protection against pulmonary edema in rats exposed to ozone or nitrogen dioxide. Other inhibitors of protein-synthesis, actinomycin D or puromycin, failed to show such effects. Possible actions of these agents as well as the doses and times that afforded the significant protection were investigated. These agents, by themselves, did not alter the water content of the lungs. In vitro study revealed that both cycloheximide and emetine hardly acted as scavengers of oxidant. Pretreatment with either agent was associated with a significant increase in the activity of glucose 6-phosphate dehydrogenase of the lungs, but the increase did not necessarily coincide with the protection. Activity levels of non-protein SH, glutathione-peroxidase and -reductase in the lungs of rats treated with either agent were scarcely altered. The effect of these agents administered in vivo or in vitro on the in vitro lipid peroxidation by air was also investigated. Other possible mechanisms of these agents responsible for the protective effect against pulmonary edema induced by oxidants were also discussed.

INTRODUCTION

Acute exposure of animals to ozone (O₃)¹⁻⁻³ or nitrogen dioxide (NO₂)⁴⁻⁻⁵ results in pulmonary edema and hemorrhage, leading to death. It is well known that reducing substances play a great role in protecting the above effects of the oxidants. For instance, it was reported that the administration of vitamin C¹⁻⁻⁶,⁷ or SH-containing substances⁸⁻⁻⁹ to animals prior to the exposure to O₃ or NO₂ made the animals resistant to it, accompanied by less pulmonary edema and mortality rate. Dietary vitamin E is known to be a protectant against oxidant-induced damage and to be effective against lipid peroxidation in lungs of vitamin E-deficient animals exposed to O₃ or NO₂.⁹⁻⁻¹⁴

It was shown that endotoxin was an effective protectant against oxygen-induced acute and chronic lung injuries.¹⁶,¹⁰ It was also shown that pretreatment with either a monoamine oxidase inhibitor, pargyline, or succinic acid provided protection against the effects of hyperbaric oxygen.²¹

Meantime, while we investigated the experimental suppression of O₃ tolerance and of cross tolerance (NO₂-O₃) by metabolic inhibitors,¹⁸ we found that some kind of agents known as inhibitors of protein-synthesis protected pulmonary edema induced by the oxidants. This paper deals with the evidences that cycloheximide or emetine prevents the formation of edema in the lungs of rats acutely exposed to O₃ or NO₂.

MATERIALS AND METHODS

The methods of O₃-exposure and of the measurements of O₃ concentration are described elsewhere.¹⁹ Desired concentration of NO₂ was pro-
duced by diluting 1% NO₂ with charcoal-filtered room air. The concentration was monitored with a chemiluminescent NO-NO₂ analyzer (Toshiba-Beckman, Tokyo, Model 951A) and occasionally checked with the Saltzman method.²⁰³

Male Wistar rats, free from specific pathogens, 6 weeks old, weighing about 180 to 200 g, were obtained from a local breeder. The animals were kept with laboratory chow and tap water ad libitum in an air-conditioned room (22-24°C) for 4-6 days before sacrifice.

Protection with the agents against effects of the oxidants. Animals were intraperitoneally administered one of the following agents dissolved in an appropriate volume of saline: cycloheximide (5 mg/kg, Boehringer Mannheim, GmbH), emetine HCl (25 mg/kg, Sigma Chem. Corp.), puromycin dihydrochloride (45 mg/kg, Sigma Chem. Corp.), actinomycin D (0.9 mg/kg, Makor Chem. Ltd., This agent was dissolved in acetone, and then diluted 10-fold with saline), reduced-glutathione (250 mg/kg, Sigma Chem. Corp.), dimethyl disulfide (18 mg/kg, This agent was dissolved in 30% ethanol in saline), or vitamin C (500 mg/kg). Immediately after the administration or 24 hr later, the animals were exposed for 3 hr to 5.6 ppm O₂ or 50 ppm NO₂ in a 1 m³ stainless steel chamber. Food and water were withheld during the exposure. Immediately after the exposure, the animals were sacrificed by exsanguination from the abdominal aorta after anesthesia with intraperitoneal pentobarbital sodium (50 mg/kg). Lungs were removed, rinsed with saline, blotted, and weighed. The dry weights of the lungs were estimated after drying for two days in an oven at 110°C to determine the relative lung water content, (wet weight – dry weight) × 100/wet weight, (%).

Measurements of protectant antioxidant activities. After the anesthesia, the animals’ lungs were perfused through the portal vein with 37°C irrigating saline by a peristaltic pump (8 ml/min) while the lungs were artificially ventilated (respirator: 70 cycles/min, tidal volume: 1.3 ml), and then exsanguinated by cutting the abdominal aorta. Chest was then opened, and the lungs were excised, washed with ice-cold saline, blotted, weighed, minced with scissors and homogenized using an ice-cold medium containing 0.15 M NaCl–5 mM Tris-HCl–1 mM EDTA, pH 7.5. The homogenate was filtered through a two-layer cheese-cloth, and the filtrate was adjusted to a 12 ml final volume per lung with the medium and used for determination of non-protein SH.²¹) The cell sap fraction of the lung homogenate was obtained by differential centrifugation finally at 105,000 × g for 1 hr, and diluted two fold with the medium and used for measurements of glutathione-peroxidase,²⁰⁹ -reductase,²⁰⁹ glucose 6-phosphate dehydrogenase,²⁰⁹ and protein.²⁵) Protection with the agents against the release of Iₐ from KI-solution caused by ozone. Several agents were compared for the capacity to inhibit the Iₐ-release which was a result of the reaction between KI and O₃. Ten milliliters of the routine O₃-absorptive solution which has been used in the neutral-buffered KI method²⁰⁹ containing one of the agents was bubbled with 5.6 ppm O₃ for 2 min at a flow rate of 0.5 l/min in an impinger and the absorbance at 280 nm was recorded. The rate of protection against the release of Iₐ from KI was calculated as follows:

\[
(A_{\text{ref}} - A_{\text{exp}}) \times 100/A_{\text{ref}},
\]

where, \(A_{\text{ref}}\) is absorbance at 380 nm of reference solution and \(A_{\text{exp}}\) absorbance at 380 nm of experimental solution.

In vitro lipid peroxidation by air: Each lung obtained from the animal sacrificed by exsanguination after the anesthesia was weighed, and homogenized in 0.15 M KCl, and the homogenate was filtrated through a two-layer cheese cloth, and 10% homogenate (weight/volume) was obtained. A 50-ml volumetric flask without a cap containing 8 ml of the homogenate was incubated at 37°C with constant shaking and an aliquot of 1 ml was successively withdrawn from it according to the scheduled interval. The aliquot was added to 1 ml of 10% trichloroacetic acid and the mixture was cooled. After the centrifugation at 3,000 rpm for 10 min, an aliquot of 1 ml of the supernatant was mixed with 1 ml of 1% 2-thiobarbituric acid sodium salt (weight/weight), and the mixture was boiled for 10 min. The absorbance at 530 nm was used as an index of lipid peroxidation.

RESULTS

The effects of the pretreatment with the agents on the pulmonary edema induced by O₃ are summarized in Table 1. Relative lung water content (RLWC) was 80.7% in the normal lung, while 85.0% in the lung of O₃-exposed animals. Cycloheximide and emetine, administered 24 hr before
Table 1. Effects of the pretreatments with agents on the pulmonary edema produced by ozone.

<table>
<thead>
<tr>
<th>Agent</th>
<th>(mg/kg)</th>
<th>O$_3$</th>
<th>RLWC* (%) (mean±SD)</th>
<th>n</th>
<th>Agent</th>
<th>(mg/kg)</th>
<th>O$_3$</th>
<th>RLWC* (%) (mean±SD)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>−</td>
<td>80.7±0.9</td>
<td></td>
<td>8</td>
<td>Cycloheximide</td>
<td>(5)</td>
<td>+</td>
<td>81.9±0.3, a,b</td>
<td>5</td>
</tr>
<tr>
<td>None</td>
<td>+</td>
<td>85.0±1.1</td>
<td></td>
<td>15</td>
<td>Emetine-HCl</td>
<td>(25)</td>
<td>+</td>
<td>84.4±1.2</td>
<td>7</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>(5)</td>
<td>−</td>
<td>80.8±0.4</td>
<td>5</td>
<td>Puromycin-2HCl</td>
<td>(45)</td>
<td>+</td>
<td>86.8±1.4</td>
<td>6</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>(5)</td>
<td>+</td>
<td>82.3±0.76, a,b</td>
<td>10</td>
<td>Actinomycin D</td>
<td>(0.9)</td>
<td>+</td>
<td>86.0±1.4</td>
<td>5</td>
</tr>
<tr>
<td>Emetine-HCl</td>
<td>(25)</td>
<td>−</td>
<td>80.3±0.4</td>
<td>5</td>
<td>Reduced-glutathione</td>
<td>(250)</td>
<td>+</td>
<td>85.2±0.9</td>
<td>8</td>
</tr>
<tr>
<td>Puromycin-2HCl</td>
<td>(45)</td>
<td>−</td>
<td>81.3±0.2</td>
<td>5</td>
<td>Vitamin C</td>
<td>(500)</td>
<td>+</td>
<td>81.5±0.9, a,b</td>
<td>9</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>(0.9)</td>
<td>−</td>
<td>80.3±0.3</td>
<td>9</td>
<td>Dimethyl-sulphide</td>
<td>(18)</td>
<td>+</td>
<td>85.2±1.8</td>
<td>9</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>(0.9)</td>
<td>+</td>
<td>85.4±2.0</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) p<0.005 against None (+), b) p<0.025 against None (−), c) p<0.005 against None (−). RLWC*: Relative lung water content calculated by the formula presented in “Materials and Methods.”

Table 2. Effects of the pretreatments with agents on the pulmonary edema produced by nitrogen dioxide.

<table>
<thead>
<tr>
<th>Agent</th>
<th>(mg/kg)</th>
<th>Time administered before exposure (hr)</th>
<th>NO$_2$</th>
<th>RLWC* (%) (mean±SD)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>−</td>
<td></td>
<td>−</td>
<td>80.9±0.5</td>
<td>6</td>
</tr>
<tr>
<td>None</td>
<td>+</td>
<td></td>
<td>+</td>
<td>83.1±1.3, a,b</td>
<td>8</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>(5)</td>
<td>0</td>
<td>+</td>
<td>81.0±0.6, a,b</td>
<td>9</td>
</tr>
<tr>
<td>Emetine-HCl</td>
<td>(25)</td>
<td>0</td>
<td>+</td>
<td>82.7±1.6a</td>
<td>8</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>(500)</td>
<td>0</td>
<td>+</td>
<td>81.4±0.5b</td>
<td>7</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>(5)</td>
<td>24</td>
<td>+</td>
<td>81.0±0.5b</td>
<td>7</td>
</tr>
<tr>
<td>Emetine-HCl</td>
<td>(25)</td>
<td>24</td>
<td>+</td>
<td>80.4±0.3, b</td>
<td>7</td>
</tr>
</tbody>
</table>

a) at least p<0.025 against None (−), b) p<0.005 against None (−), c) p<0.05 against None (−). RLWC*: Relative lung water content.

the exposure, significantly rendered the lungs less damaged by O$_3$-exposure, resulting in 82.3% and 81.9% RLWC, respectively, although these values were slightly higher than those of the animals not-exposed, not-administered. Both agents themselves appeared not to change RLWC of unexposed rats. Actinomycin D and puromycin did not seem to affect RLWC in both O$_3$-exposed and unexposed rats. On the other hand, the immediate administration before the exposure resulted in significant decrease of RLWC in the case of cycloheximide, where RLWC was 81.9% but not in the case of emetine. Puromycin and actinomycin D administered immediately before the exposure were also not effective on decreasing RLWC in O$_3$-exposed rats. Among the reducing agents tested, only vitamin C administered immediately before the exposure significantly decreased RLWC to 81.5% in the O$_3$-exposed rats.

Protection against effects of NO$_2$ with the agents is presented in Table 2. The RLWC in the normal rat in this experiment was 80.9%, while it was 83.1% in NO$_2$-exposed rats. Administration of cycloheximide and emetine 24 hr before the exposure significantly prevented the increase in RLWC of NO$_2$-exposed rats, resulting in 81.0% and 80.4% RLWC, respectively. Immediate pretreatment with cycloheximide and vitamin C also significantly protected the effects of NO$_2$, resulting in 81.0% and 81.4% RLWC, respectively, but emetine appeared not to be effective. These results are compatible with those obtained in the case of the exposure to O$_3$.

As shown respectively in Figs. 1 and 2, the protective effects of cycloheximide and emetine against pulmonary edema produced by O$_3$ were investigated in view of a dose-effect relationship. In the case of cycloheximide administered immedi-
ately before the exposure to O$_3$, a significant decrease of pulmonary edema was noticed by the dose of 0.5 mg/kg, and it required more cycloheximide than the dose of 1.25 mg/kg to fully protect the development of pulmonary edema. When it was administered 24 hr before the exposure, the dose of 0.5 mg/kg was not effective, and the full protection was obtained with the administration of doses over 2.5 mg/kg. In the case of emetine administered 24 hr before the exposure, it required more emetine than the dose of 12.5 mg/kg to fully protect the pulmonary edema produced by O$_3$.

The rates of protection with the agents against the release of I$_2$ from the KI-solution caused by O$_3$-bubbling are shown in Table 3. Cycloheximide (1 mg/10 ml) scarcely prevented I$_2$-release from the KI-solution. Emetine (1 mg/10 ml) appeared to show a moderate protective effect against the I$_2$-release, but the solution became less yellow when it was left standing after the bubbling, suggesting that the successive addition of the released I$_2$ to the unsaturated bonds in emetine might occur. Both reducing substances, reduced-glutathione (10 mg/10 ml) and vitamin C (10 mg/10 ml), almost completely protected the I$_2$-release from the solution.

**Protectant antioxidant activities**

As presented in Table 4, the protectant antioxidant activities, such as non-protein SH, glutathione-peroxidase, -reductase and glucose 6-phosphate dehydrogenase, in the lungs of normal rats were compared with those in the lungs of animals administered cycloheximide or emetine. The activi-

![Fig. 1. Dose-effect relationship between cycloheximide administered before the exposure and pulmonary edema induced by ozone. In this case, we presented lung weight as an index of pulmonary edema instead of RLWC, because the absolute value of the lung should be cited somewhere in this paper. ○: Lung wet weight of rats administered cycloheximide (0 to 5 mg/kg) immediately before the exposure to 5.6 ppm ozone for 3 hr. ●: Lung wet weight of rats administered cycloheximide (0 to 5 mg/kg) 24 hr before the exposure to 5.6 ppm ozone for 3 hr. C: Control animals administered saline at an appropriate time. All values presented are means±SD of 4 to 8 rats.](image1)

![Fig. 2. Dose-effect relationship between emetine administered before the exposure and pulmonary edema induced by ozone. ○: Lung wet weights of animals administered emetine (0 to 25 mg/kg) 24 hr before the exposure to 5.6 ppm ozone for 3 hr. C: Control animals administered saline. All values are means±SD of 4 to 6 rats.](image2)

**Table 3. Rates of protection with agents dissolved in KI-solution for the measurement of ozone against the release of I$_2$ from the KI-solution by ozone-bubbling.**

<table>
<thead>
<tr>
<th>Agent</th>
<th>(mg/10 ml)</th>
<th>Time after the bubbling (min)</th>
<th>Rate of protection against I$_2$-release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>1</td>
<td>0</td>
<td>3.5</td>
</tr>
<tr>
<td>Emetine-HCl</td>
<td>1</td>
<td>0</td>
<td>20.1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5</td>
<td>49.9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10</td>
<td>66.0</td>
</tr>
<tr>
<td>Glutathione</td>
<td>10</td>
<td>0</td>
<td>96.6</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>10</td>
<td>0</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table 4. Protectant antioxidant activities.

<table>
<thead>
<tr>
<th>Agent (hours after the administration)</th>
<th>N</th>
<th>BW (g)</th>
<th>LWW (g)</th>
<th>Non-protein SH (µmoles/lung)</th>
<th>GPO (µmoles NADPH/min/mg protein)</th>
<th>GR</th>
<th>G6PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8</td>
<td>228±3</td>
<td>1.00±0.03</td>
<td>2.23±0.11</td>
<td>45.38±5.36</td>
<td>25.58±2.67</td>
<td>28.87±1.22</td>
</tr>
<tr>
<td>Cycloheximidea) (2-4 hr)</td>
<td>6</td>
<td>228±4</td>
<td>1.01±0.07*</td>
<td>2.39±0.17</td>
<td>46.74±7.36</td>
<td>25.45±2.15</td>
<td>**34.71±3.53</td>
</tr>
<tr>
<td>Emetineb) (2-4 hr)</td>
<td>6</td>
<td>232±4</td>
<td>**<em>1.11±0.08</em></td>
<td>***2.49±0.14</td>
<td>47.87±4.68</td>
<td>25.74±1.52</td>
<td>***34.76±3.11</td>
</tr>
<tr>
<td>Cycloheximidea) (24 hr)</td>
<td>5</td>
<td>230±5</td>
<td>1.08±0.10</td>
<td>2.23±0.12</td>
<td>46.37±3.25</td>
<td>25.05±2.08</td>
<td>***36.89±3.60</td>
</tr>
<tr>
<td>Emetineb) (24 hr)</td>
<td>6</td>
<td>227±6</td>
<td>**<em>1.14±0.07</em></td>
<td>**2.47±0.21</td>
<td>50.41±5.72</td>
<td>26.45±1.83</td>
<td>***35.71±1.59</td>
</tr>
</tbody>
</table>

a) 5 mg/kg, b) 25 mg/kg, * number of rats=5, ** p<0.025, *** p<0.005.

Fig. 3. In vitro lipid peroxidation by air. Experimental details are presented in “Materials and Methods.” Each value is the mean of 4 to 5 rats. *Significantly different (p<0.05) from normal rat lungs. ◯ : lungs of rats administered emetine (25 mg/kg) 24 hr before sacrifice, ● : lungs of rats administered emetine (25 mg/kg) 2-4 hr before sacrifice, □ : lungs of rats administered cycloheximide (5 mg/kg) 24 hr before sacrifice, ■ : lungs of rats administered cycloheximide (5 mg/kg) 2-4 hr before sacrifice. x : normal rat lungs.

Figures and text describe the results of experiments on the effects of various compounds on antioxidant activities. The content of non-protein SH as well as the weight of the lungs among the five groups were nearly similar to each other, although there were some significant differences. Both agents made the animals administered either of them have less appetite, resulting in no increase of body weight 24 hr after the administration.

In order to elucidate possible involvement of the agents in lipid peroxidation, in vitro lipid peroxidation by air of the lung homogenate obtained from normal rats or rats administered emetine or cycloheximide was investigated. As shown in Fig. 3, however, there seems to be little difference among the measurements for the five groups of rats. On the other hand, preliminary studies revealed that in vitro administration to the normal rat lung homogenate resulted in no change in the in vitro lipid peroxidation by air in the case of cycloheximide (final concentration: 100 µg/ml), but the depression of the in vitro lipid peroxidation by air in the case of emetine (final concentration: 500 µg/ml) and vitamin E (final concentration: 1 µmol/ml).

**DISCUSSION**

In the present study, we demonstrated that treatment with cycloheximide or emetine resulted in a marked change in the susceptibility of young adult rats to the acute exposure to O₃ or NOₓ. Appropriate doses of these agents markedly ameliorated pulmonary edema induced by either oxidant. These agents have been known as inhibitors of protein-synthesis, and as inducers of fatty liver.⁷,²⁶³ Cycloheximide, of which another name is actidione, is extremely repellent to rats, while emetine has been used as an amebicide and for lung worm infection.

Possible properties responsible for this protec-
tive effect against pulmonary edema have been investigated in reference to an action of scavenger, such as vitamin C, an action of activator of antioxidant activities, such as endotoxin, and an action of antioxidant like vitamin E.

The in vitro results in Table 3 suggested that glutathione or vitamin C could act as a competitor with KI, that is, as a scavenger of oxidants, but that cycloheximide or emetine, as far as with the doses used in the in vivo experiments, hardly acted as a scavenger of oxidants. Supposing that exogenous scavenger of oxidant will not maintain its activity so long after the administration, the suggestion described above is also supported by the evidences that both agents administered 24 hr before the exposure protected the formation of pulmonary edema, and by the evidence that emetine injected just before the exposure failed to provide the protection.

Pretreatment with either agent was associated with scarce change in the pulmonary activity levels of non-protein SH, glutathione-peroxidase and -reductase, but with increased activity of glucose 6-phosphate dehydrogenase. However, this latter response might be rather correlated to a possible catabolism of these agents than to a protective effect against pulmonary edema, because its increase did not necessarily coincide with the protection as follows: the administration of emetine just before the 3-hr exposure to either oxidant did not protect the significant increase in pulmonary edema produced by the exposure, and nevertheless, the lungs of animals left standing 2–4 hr after the administration of emetine showed the significant increase in glucose 6-phosphate dehydrogenase activity, and the extent of the increase was as much as those in other cases of the administration. These responses of the antioxidant activities are quite different from those obtained by the endotoxin treatment. It was reported\(^\text{15,16}\) that the endotoxin treatment significantly increased the activities of lung superoxide dismutase, catalase, and glutathione-peroxidase.

The suppressive effect of vitamin E on lipid peroxidation is well known,\(^\text{8,14}\) and such an effect was suggested by the present study on the in vitro lipid peroxidation by air. It was also found that the in vivo administration of cycloheximide or emetine did not alter the rate of the in vitro lipid peroxidation by air. It is unknown why the in vitro administration of emetine resulted in the depression of the in vitro lipid peroxidation by air. However, at least, it seems that the in vivo effect of cycloheximide or emetine against the effects of O\(_2\) or NO\(_2\) is not ascribed to a depression of lipid peroxidation as expected in the case of the administration of vitamin E.

Unfortunately, we are presently unable to present any evidence which explains a possible mechanism of the action of cycloheximide or emetine, but we can speculate that some of the previously known biological actions of either agent might be important in explaining their demonstrated protective effects against pulmonary edema produced by O\(_2\) or NO\(_2\).

Cycloheximide and emetine are known\(^\text{29}\) to inhibit protein synthesis through interaction with 60 S eukaryotic ribosomal subunit and through stabilization of 80 S eukaryotic ribosomes, respectively. It was reported that the active structure of cycloheximide was similar to that of emetine, and that the similarity of action of them were evident.\(^\text{29}\) These events suggest possible correlation between ribosomes and edema, but the gap to be filled is still too deep. Ribosomes stabilized with cycloheximide or emetine might be stable to free radicals or oxidants, leading to a less severe pulmonary edema.

In addition to the inhibitory effect on the protein biosynthesis, the administration of cycloheximide or emetine may result in a certain change of physiological conditions in the lungs, directly or indirectly through a sort of intermediator, so that the agent can play as an antioxidant. In this regard, it is worthy of note that both endotoxin and inhibitors of protein-synthesis, such as cycloheximide, have been known as effective interferon-inducers.\(^\text{20}\) Endotoxin was also reported\(^\text{15,16}\) to provide certain protection against oxygen toxicity. Tilorone has also been just reported as an effective protectant against oxygen toxicity\(^\text{31}\) as well as an effective interferon-inducer.\(^\text{30}\) It may be interesting to speculate a possible relation between the antioxidant activity and the production of interferon. However, the mechanisms to explain the decreased susceptibility of the lungs to O\(_2\) or NO\(_2\) are still unknown, and it is uncertain whether there is a common mechanism between cycloheximide and emetine. But these agents may be good tools for investigation of edema induced by oxidants.

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和文要旨
オゾン (O₃) または二酸化窒素 (NO₂) による肺水腫を防ぐ薬剤として、ビタミン C, E および・SH 含有物質等の抗酸化剤が知られている。酸素による肺水腫を抑える薬剤として菌体内毒素サリゲンオキシダーゼの阻害剤であるパルギリン (pargyline) も最近知られてきた。われわれはタンパク合成の阻害剤として知られるシクロヘキシミドとアンチビタミン C または NO₂ による肺水腫形成を防ぐことを認めたので報告する。

JCL-Wistar 系雄ラット（220～240 g, 7 週齢）に以下の薬品、 cycloheximide（5 mg/kg 体重, 以下同じ）、 emetine · 2HCl (25), puromycin · 2HCl (45), actinomycin D (0.9), glutathione (250), vitamin C (500), di-
methyldisulfide (18) をおのおの胸腔内注射し、ただちにあるいは 24 時間後よりこれらのラットを 3 時間 O₃ (5.6 ppm) または NO₂ (50 ppm) に暴露し、暴露直後に麻酔し、卵火し、肺重量を測定した。O₃ 暴露実験の場合、正常肺の相対水分量は 80.7％、暴露肺で 85.0％ であった。シクロヘキシミドまたはアンチビタミンを投与されてから 24 時間後には NO₂ 暴露されたラットのそれはおのおの 82.3％ ＆ 81.9％ であり、有意 (p<0.005) に肺水腫の形成は抑えられた。しかしこれらの中は正常肺 (80.7％) に対しては有意に増加していた。アクチノマイシン D またはピューロマイシンには、上記の効果は認められなかった。用いた四種の阻害剤は肺水腫形成作用はなかった。

O₃ 暴露直後の投与の場合は、シクロヘキシミドとビタミン C にのみ抑制効果を認めた。NO₂ 暴露実験の結果は O₃ 暴露実験の結果とはほぼ一致していた。投与量－効果曲線を O₃ 暴露について求めた。

以上の両薬剤の作用機序を①ビタミン C のような抗酸化作用、②菌体内毒素のような抗酸化系活性値の上昇作用、③ビタミン E のような抗脂質過酸化能の点より調えたが、いずれも当を得なかった。他の予測される可能性について次に論じた。シクロヘキシミドとアンチビタミンは肺水腫形成の機序をさぐる良い道具となると思われる。

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