EFFECT OF OXYGEN CONCENTRATION ON PULMONARY FIBROSIS CAUSED BY PEPLOMYCIN IN MICE

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The pulmonary fibrosis caused by peplomycin (PEP) was studied in terms of oxygen toxicity using ICR mice. When 16 μg of PEP was administered intratracheally in mice after exposure to the air containing 75% O_2 for 10 days, the pulmonary fibrosis was completely suppressed, while when mice were exposed to 75% O_2 after the administration of PEP, the fibrosis was much severe than that of mice raised in atmospheric air. In 50% O_2, similar oxygen effect was also observed, but it was weaker than that in 75% O_2. In 90% O_2, the oxygen toxicity was observed in mice without administration of PEP.

When mice were exposed to 75% O_2, the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, which are relevant to the detoxication of active oxygen species, were not increased in the lung, but the levels of reducing agents such as glutathione and ascorbic acid, and high molecular substances having $^1O_2$-scavenging activity were enhanced. The results suggest that these materials have some roles to decrease the pulmonary fibrosis caused by PEP.

Peplomycin (PEP) is one of new biosynthetic bleomycins (BLMs), which were produced by the culture of BLM-producing microorganism in the presence of artificial amines[1], and has been used clinically in Japan since 1981. Although PEP shows a less pulmonary toxicity[2,3] and a stronger antitumor activity[4] than BLM clinically used at present and is also effective against prostatic and breast cancers[5] which are insensitive to the BLM, its clinical use is still limited by the pulmonary fibrosis.

The primary biological action of BLM appears to be oxidative DNA cleavage caused by the ternary complex: BLM-Fe(III)-O_2^{2-} [6]. Clinical studies have suggested that the pulmonary toxicity caused by BLM was enhanced when the patients were exposed to a higher concentration of oxygen[7]. Therefore, we have studied the effect of oxygen concentration in aeration on pulmonary fibrosis caused by PEP in mice.

Materials and Methods

Chemicals and Animals

PEP was prepared at Nippon Kayaku Co., Ltd., Japan. Superoxide dismutase (SOD) (2,700 U/mg protein), catalase (2,000 U/mg protein), glutathione (GSH) reductase (160 U/mg protein) and linolenic acid (approximately 99% pure) were purchased from Sigma Chemical Co., USA. All other chemicals were of reagent grade. Male 14 weeks-old ICR mice (S.P.F.) were obtained from Shizuoka Agricultural Association for Laboratory Animals, Japan, and the 15 weeks-old mice were used in the experiments.

Experimental Schedule to Expose to Oxygen

The O_2 exposure experiment was conducted in a polyvinyl chloride chamber of about 0.15 m^3. The artificial O_2-rich air was prepared by mixing of oxygen, nitrogen and a trace of carbon dioxide

(*ca.* 0.5%) and was adjusted to 40~50% relative humidity at 24±2°C. The oxygen concentration was intermittently monitored by a Ventronics Oxygen Monitor, USA. Mice were exposed to the airs of 50, 75 and 90% O_2 contents in a given period before or after administration of PEP while control mice were raised in atmospheric air. The air was exchanged by 2 volumes per hour. Mice were allocated into 4 or 5 groups. The first group was exposed to the O_2-rich air before administration of PEP (1 or 2 exposure period levels), the second was exposed to the O_2-rich air after the administration of PEP, the third (control) was raised in atmospheric air throught the experiment, and the fourth was raised in the O_2-rich air without administration of PEP to test the toxicity of the O_2-rich air. Sixteen μg of PEP in 50 μl of saline was instilled intratracheally in mice under light anesthesis.

Tissue Preparation for Morphological Evaluation of Pulmonary Fibrosis

The mice were sacrificed 4 weeks after PEP treatment. The lobes were fixed with 10% formalin solution, embeded in paraffin, processed and stained with both hematoxylin and eosin, and azan and mallory. The pulmonary fibrosis was evaluated by the procedure previously reported[8].

Preparation of Lung Homogenate

The lungs of mice sacrificed by exsanguination were washed with cold saline to remove intravascular blood as much as possible. After removal of tracheobronchial and vascular tissues, the excised lungs were minced and homogenized in approximately 20 volumes of 0.1 M phosphate buffer containing 5 mM EDTA (pH 7.4) with a Potter-Elvehjem Tefron pestle. After centrifugation at 12,000×g for 20 minutes at 0°C, the supernatant, of which the protein content was measured by LOWRY's method[9], was used as the lung homogenate.

Assay of Enzymes

The SOD activity of the lung homogenate was determined by the inhibition of reduction of nitroblue tetrazolium chloride by superoxide anion ($O_2^{\bar{\cdot}}$)[10]. The $O_2^{\bar{\cdot}}$ was supplied by xanthine-xanthine oxidase reaction. The activities of GSH peroxidase and reductase were measured by LITTLE's and HORN's method, respectively[11,12]. The catalase activity was determined by COHEN's method[13].

Measurement of Singlet Oxygen($^1O_2$)-scavenging Activity

The singlet oxygen-scavenging activity of the lung homogenate was measured according to the method of KELLOG and FRIDOVICH, which is based on inhibition of peroxidation of linolenate by $^1O_2$ produced photochemically[14].

Biochemical Analyses

The reduced glutathione (GSH) in the lung homogenate was determined by spectrometry (342 nm) of the reaction product of 2,2′-dithiodipyridine with the GSH by the method of GRASSETTI[15]. The total ascorbic acid was determined by colorimetry of the condensation product with 2,4-dinitrophenylhydrazine after oxidation of reduced ascorbic acid by bromine[16].

Results

Table 1 shows the effect of oxygen concentration on the pulmonary fibrosis caused by PEP. When 16 μg of PEP was instilled intratracheally after exposure to an O_2-rich air, the pulmonary fibrosis was reduced, and when PEP was instilled after exposure to 75% O_2 for 10 days, the pulmonary fibrosis was completely suppressed. On the other hand, when the mice were exposed to 75% O_2 after the instillation of PEP, the fibrosis was much severe than that of the PEP-treated mice raised in atmospheric air. In 90% O_2, the pulmonary fibrosis was observed in mice without administration of PEP, but in 50 and 75% O_2, the oxygen toxicity was not observed.

The activities of SOD, GSH peroxidase, GSH reductase and catalase, which are relevant to detoxication of active oxygen species, in the lung homogenate of mice exposed to 75% O_2 were measured. As shown in Chart 1-A, these enzyme activities were not changed significantly during the exposure

Table 1. Effect of oxygen concentration on pulmonary fibrosis caused by peplomycin.

O_2 (%)	No. of mice	Schedule (weeks)	Grade of fibrosis[b]
		(For 50% O_2 toxicity)	
	5		0/15 = 0.00
	5	a (Control)	14/15 = 0.93
50	5	−11 days	10/15 = 0.67
	5		18/15 = 1.20
		(For 75% O_2 toxicity)	
	5		0/15 = 0.00
	5	(Control)	15/15 = 1.00
75	5	−5 days	5/15 = 0.33
	5	−10 days	0/15 = 0.00
	5		39/15 = 2.60
		(For 90% O_2 toxicity)	
	9		18/27 = 0.67[c]
	4	(Control)	14/12 = 1.17
90	5	−5 days	7/15 = 0.47
	4	−9 days	2/12 = 0.17
	4		35/12 = 2.92[d]
		0 1 2 3 4	

Oxygen chamber, ____ Atmospheric air

[a] Administration of PEP (16 μg/50 μl).

[b] See ref 8 for histopathological evaluation of the pulmonary fibrosis.

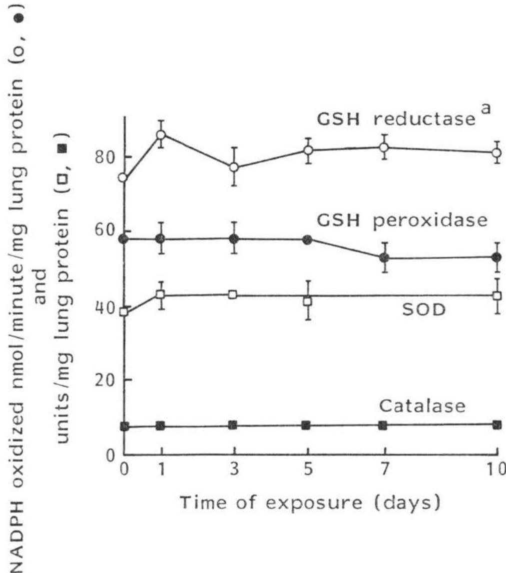
[c] Fifteen mice were exposed to 90% O_2 for 3 weeks. Nine survived mice after 4 weeks were evaluated for the fibrosis.

[d] Six of ten mice died. Four survived mice were evaluated for the fibrosis.

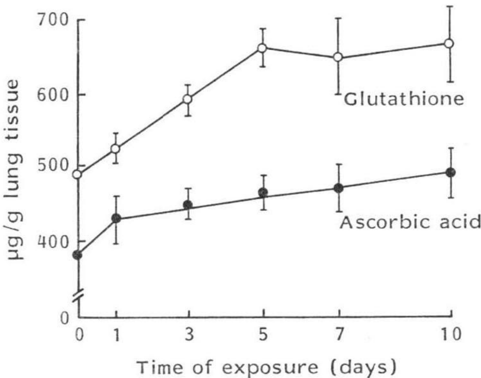
Chart 1.

A. Effect of 75% O_2 on the levels of enzymes relevant to detoxication of active oxygen species in the lung homogenate of mice.

[a] Each value shows mean±S.D.



B. Effect of 75% O_2 on the levels of glutathione and ascorbic acid in the lung homogenate of mice.



period. The levels of GSH and ascorbic acid in the lung homogenate of mice exposed to 75% O_2 were increased with the exposed period. After 10 days of the exposure, the levels of GSH and ascorbic acid were increased by 45 and 30%, respectively (Chart 1-B). The $^1O_2$-scavenging activity in the lung homogenate of mice exposed to 75% O_2 was also increased with the exposed period. This $^1O_2$-scavenging activity did not change after dialysis or treatment at 56°C for 30 minutes (data are not shown).

Discussion

Although the pulmonary changes induced by BLM have been studied extensively from ultrastructural[17], physiological[18], biochemical[19,20] and immunological[21] viewpoints, the process of the damage of lung cells caused by BLM is not yet clearly elucidated. GOLDINER *et al.* reported that the pulmonary toxicity induced by BLM is enhanced when the cancer patients are exposed to a higher concentration of oxygen[7], and TRYKA *et al.* also reported that the BLM-induced pulmonary toxicity is potentiated by oxygen in hamster[22]. We have studied the pulmonary fibrosis caused by PEP in terms of oxygen toxicity using ICR mice and found that PEP-induced pulmonary fibrosis was enhanced by exposure to an O_2-rich air after administration of PEP while it was reduced by exposure to the O_2-rich air before the administration (Table 1). The pulmonary damage was also observed when the mice were exposed to 90% O_2 without administration of PEP (Table 1). ADAMSON *et al.* reported that the type I cells of lung were damaged when mice were exposed to a higher concentration of oxygen[23]. The morphological changes of the lung caused by PEP were similar to those exposed to 90% O_2 alone (the photos are not shown). Oxygen toxicity caused by a higher concentration of oxygen may be explained by increase of active oxygen species such as superoxide anion, hydroxyl radical and peroxides[24,25]. The active form of BLM appeare to be the ternary complex: BLM-Fe(III)-O_2^{2-}[6]. This complex containing an active oxygen should give the pulmonary damage as the active oxygen species do. Therefore, we have studied the relationships between the pulmonary toxicity caused by PEP and the activities of enzymes relevant to detoxication of active oxygen species and between the pulmonary toxicity and the levels of reducing agents such as GSH and ascorbic acid, and high molecular substances[26] having $^1O_2$-scavenging activity.

The enzymes relevant to detoxication of active oxygen species include SOD, catalase, GSH peroxidase and GSH reductase. GREGORY and FRIDOVICH observed that SOD was induced in microorganism[27] by molecular oxygen, and KIMBALL *et al.* also reported that the levels of SOD, GSH peroxidase and GSH were increased by exposure to 85~90% O_2 in rat[28]. In our experiment using mice, the levels of SOD, catalase, GSH peroxidase and GSH reductase did not change by exposure to 75% O_2 (Chart 1-A). It was reported that ICR mice are susceptible to BLM-induced pulmonary fibrosis while Wistar rats are resistant[5]. This may be due to the difference in activities of these enzymes.

The levels of ascorbic acid, GSH and $^1O_2$-scavenging materials were increased with the exposure time to 75% O_2 (Chart 1-B). Previously we reported that *dl*-α-tocopherol (a $^1O_2$-scavenger)[29], ascorbic acid and GSH[30] suppressed the pulmonary fibrosis caused by PEP. The suppression of the pulmonary fibrosis by the exposure to 75% O_2 before the administration of PEP may be due to the augmentation of reducing agents such as ascorbic acid and GSH, and $^1O_2$-scavenging materials.

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