Short Communication

EFFECTS OF EXPOSURE TO 0.20 ppm
OZONE FOR SIX WEEKS ON
EMPHYSEMATOUS RATS肺気腫ラットにおよぼす 0.20 ppm オゾン
6 週間暴露の影響*Key words:* ozone; pulmonary emphysema.

Ozone (Oz) is one of the potent air pollutants in photochemical smog. Most of the accumulated knowledge regarding the toxicity of Oz has been obtained through studies with normal laboratory animals and normal humans. The human population, however, is composed of not only normal people but also those in poor health. From the viewpoint of public and industrial health, it is better to study the effects of air pollution on people in poor health having a chronic obstructive pulmonary disease. In this context, we have previously reported tolerance to ozone in emphysematous rats.¹⁾

This paper deals with biochemical responses of an experimental model of pulmonary emphysema in rats to subchronic exposure to low level of Oz in order to obtain information for evaluating whether this model is more susceptible to atmospheric pollutants than a healthy group.

Materials and methods. Specific pathogen free, 8-wk-old male Wistar rats were purchased and divided into four groups as follows; saline-administered and air-exposed, S-A; saline-administered and Oz-exposed, S-Oz; elastase-administered and air-exposed, E-A; and elastase-administered and Oz-exposed, E-Oz. Some of the animals were intratracheally administered porcine pancreatic elastase (6.5 unit/rat), kept for 6 wk to obtain pulmonary emphysema and exposed to 0.20 ppm Oz for another 6 wk. Control rats were administered saline alone, and exposed to pre-, HEPA- and charcoal-filtered air. Detailed procedures for making a model of pulmonary emphysema in rats are available elsewhere.^{1,2)} For the exposure, two stainless steel chambers of 590 l were used; one for the exposure to Oz and the other for the control experiment. They were ventilated at 15 times/h under a pressure slightly lower than 1 atm by top to bottom uni-directional flow of pre-, HEPA- and charcoal-filtered air.

Oz was generated by a silent electrical discharge Oz generator from the air desiccated with a heatless drier, led to a mixing chamber, diluted by air and introduced to the exposure chamber. Concentration of Oz in the chamber was continuously monitored by a UV absorbance ozone analyzer, and the concentration was automatically regulated by a system using a mass flow controller. After exposure, the animals were anesthetized with intraperitoneal injection of sodium pentobarbital, and after obtaining blood with a heparinized syringe from the abdominal aorta, the rats were exsanguinated. The lungs of the rats were excised, rinsed with saline, blotted and weighed. They were then homogenized in 16 ml of a buffer containing 0.15 M NaCl-1 mM EDTA-5 mM Tris-HCl (pH 7.5). Concentrations of non-protein SH in an aliquot of the homogenate and blood were measured by the method of Du Lucia et al.³⁾ Another aliquot of the homogenate was centrifuged at 3,000 rpm for 10 min to obtain a precipitable fraction. After the fraction was treated according to Lancing-procedure,⁴⁾ the quantity of elastin, that is, insoluble residue and collagen solubilized, was determined by a gravimetric method and hydroxyproline measurement,⁵⁾ respectively.

Results and discussion. Results are summarized in Table 1. Although it has been known that exposure of rats to 0.25 ppm Oz for 2 wk decreased food intake and growth rate, and that further 2 wk of exposure caused the rats to lose body weight,⁶⁾ it was found that body weights of the rats were not different from each other, suggesting that they were not affected by the present exposure to 0.20 ppm Oz. Consequently, it seems that the critical concentration of Oz for rats to grow is between 0.25 and 0.20 ppm. Lung wet weight per body weight (LWW/BW) of the E-A group was found to be significantly greater than that of the S-A group, which is compatible with our previous results.^{1,2)} Lung wet weight of the E-Oz group was significantly heavier than that of the others, and in both the saline and elastase-administered groups, LWW/BW of the Oz-exposed group was significantly greater than that of the air-exposed group, suggesting that the lungs of the Oz-exposed groups were similarly edematous to some extent.

It was found that, in the case of saline-administered rats, the exposure to Oz significantly en-

Table 1. Biochemical effects of exposure to ozone on emphysematous rats.

No. of animals used	Saline-administered (4)		Elastase-administered (5)	
	Air-exposed	Ozone-exposed	Air-exposed	Ozone-exposed
Body weight (BW)	395 ± 18	374 ± 14	386 ± 32	401 ± 29
Lung Wet Weight (LWW)	1.17 ± 0.03	1.20 ± 0.06	1.23 ± 0.05	1.36 ± 0.04 ^{*,##}
(LWW/BW) × 1,000	2.96 ± 0.06	3.21 ± 0.14 [*]	3.18 ± 0.14 [‡]	3.41 ± 0.15 [*]
Non-Protein SH in Lung Homogenate (μ moles/Lung)	2.57 ± 0.13	2.81 ± 0.11 [*]	2.82 ± 0.21	3.05 ± 0.21
(μ moles/LWW)	2.20 ± 0.05	2.34 ± 0.05 ^{**}	2.30 ± 0.09	2.24 ± 0.13
Non-Protein SH in Blood (μ moles/ml)	1.24 ± 0.06	1.00 ± 0.10 ^{**}	1.18 ± 0.02	0.96 ± 0.09 ^{**}
Elastin (mg/Lung)	2.4 ± 0.3	2.9 ± 0.4 [‡]	2.8 ± 0.5	4.0 ± 0.5 ^{*,##}
(mg/LWW)	2.0 ± 0.2	2.5 ± 0.3 [‡]	2.3 ± 0.4	2.9 ± 0.3 [*]
Collagen (mg/Lung)	18.2 ± 2.8	21.7 ± 1.5 [‡]	20.8 ± 3.8	26.2 ± 3.2 ^{*,‡}
(mg/LWW)	15.5 ± 2.0	18.1 ± 1.6 [‡]	16.9 ± 2.7	19.2 ± 2.4

‡ : Four animals were used. *, ‡ : Significantly different ($p < 0.05$) from air-exposed and saline-administered, respectively. **, ## : The same as described above but $p < 0.01$.

hanced the content of non-protein SH in the lung homogenate and decreased it in the blood. However, in the case of elastase-administered rats, the exposure to Oz did not affect the contents in the lung homogenate and significantly decreased it in the blood. It is very interesting to note that the content of non-protein SH in the lung homogenate of the elastase-administered rats did not increase by exposure to Oz, because an increment of non-protein SH in lung homogenate of normal laboratory animals due to an exposure to Oz has been accepted to be a sensitive index of chronic exposure to Oz. It is noteworthy that the content of non-protein SH in the blood decreased simultaneously due to exposure to Oz in both cases, suggesting its participation in the formation of pulmonary edema.

Contents of elastin and collagen per lung of the E-Oz group were found to be significantly higher than those of the E-A and the S-Oz groups. Even when the content was normalized by lung wet weight, the content of elastin in the E-Oz group was significantly higher than that of the E-A group. These results suggest that emphysematous rats are much more responsive to exposure to Oz in elastin and collagen metabolism than normal rats.

It is worthy of note that biochemical responses of emphysematous rats to Oz were found to be somewhat different from those of normal rats. However, from the results described above, it is difficult at present to evaluate whether exposure to Oz constitutes a greater risk for emphysematous rats than for normal rats. Further experiments

are needed to gather more information regarding the subject before making the evaluation.

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