Accumulation of (E)-4-Hydroxy-2-nonenal and n-Hexanal, Degradation Products of Lipid Peroxides, in Mouse Lung and Liver

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Effects of lipid peroxide breakdown products, (E)-4-hydroxy-2-nonenal (4-HN) and n-hexanal, on mouse lung lesion were examined. When 4-HN was injected i.v., the plasma level of 4-HN increased just after the injection and then decreased immediately. The amounts of 4-HN increased in the liver and lung were ca. 0.085 and 0.43% to the dose administered, respectively, 5 min after the injection. Reduced glutathione (GSH) content and both GSH peroxidase (GSH-Px) and GSH reductase (GSSGR) activities in the lung were decreased significantly by 4-HN treatment. On the other hand, in the case of i.v. injection of n-hexanal into mice, the amount of n-hexanal detected in the lung was 5.0% to that of 4-HN, and no effect on the activities of GSH-Px and GSSGR and the content of GSH was observed.

These results suggest that 4-HN generated from lipid peroxides would be transferred into the lung and cause the lung lesion through the inhibition of GSH-dependent antioxidative defense systems.

Keywords 4-hydroxy nonenal; n-hexanal; mouse lung; glutathione peroxidase; glutathione reductase; glutathione

(E)-4-Hydroxy-2-nonenal (4-HN), n-propanal, and n-hexanal were formed from peroxidative products of ω-6 unsaturated fatty acid by air oxidation and xanthine-xanthine oxidase lipid peroxidation.2,3 These aldehydes have been reported to increase in the liver and plasma from rats fed the vitamin E deficient diet or in the plasma of rats following oral administration of carbon tetrachloride.4,5

Among these aldehydes, 4-HN is known to produce strong deleterious effects on the cell membrane and various enzymes such as glucose 6-phosphatase (EC 3.1.3.9) or cytochrome P-450.6,7,9,10 Recently, it has been reported that 4-HN causes chemical modification of low density lipoproteins and could enhance their accumulation in monocyte-macrophages.11

On the other hand, in in vivo experiment, n-hexanal was detected temporarily in rat breath during iron-dextrane-induced liver lipid peroxidation.12 This fact suggests that at least an aliquot of n-hexanal could be removed from the liver into the lung and eliminated in expired gas. In the case of 4-HN, however, little is known about its fate such as its movement into the lung and the expiration into breath. If 4-HN produced in various tissues is transferred into the lung and accumulated, it may cause serious effects on lung tissue, since 4-HN has deleterious effects on the cells and has low volatility.

In this study, we examined the accumulation of 4-HN or n-hexanal in mouse lung and the damage on SH-enzymes when each authentic aldehyde was injected intravenously.

Experimental
Chemicals Cyclohexane-1,3-dione (CHD) was purchased from Aldrich Chemical Co. and was purified by recrystallization from MeOH. MeOH used was distilled after the treatment with 2,4-dinitrophenyl hydrazine. 4-HN was synthesized using n-nonanal obtained from Wako Pure Chemicals, according to the report of Esterbauer and Weger.13 The purity of 4-HN was ca. 96.5% as the result of the determinations by 1H-nuclear magnetic resonance and gas chromatography. n-Hexanal was purchased from Wako Pure Chemicals, nicotinamide adenine dinucleotide phosphate was obtained from Oriental Yeast Co., and other chemicals used were of reagent grade quality.

Apparatus For the determination of the aldehydes, a Spectra-Physics high-performance liquid chromatography (HPLC) with a Hitachi F-1000 HPLC fluorescence detector was used. As the HPLC column, an ERC-ODS-1262 column (5 μm, 10 × 0.6 cm; Erma Optical Works Ltd.) was employed.

Animals Six-week-old male ddY mice (Clea Japan Inc.) were used and were fed a commercial laboratory diet (CE-2; Clea Japan Inc.) and given tap water ad libitum. They were fasted for 15 h before the administration of the aldehyde, n-hexanal or 4-HN was dissolved in physiological saline containing 1% bovine serum albumin and administered to mice at a dose of 6.8 μmol/10 g body weight by i.v. injection into the tail vein.

Analysis Mice administered the aldehydes were dissected under ether anesthesia at 0, 5, 10, 30, 60, and 120 min after the treatments. Blood was drawn from the heart and the plasma was prepared by centrifugation. The liver and lung were removed after perfusion with ice-cold physiological saline and were homogenized in 9 volumes of 40 mM phosphate buffer (pH 7.4). The contents of n-hexanal or 4-HN in the plasma and tissue homogenates were determined by CHD-HPLC fluorometric assay as described in the previous paper.14

The lung homogenates were centrifuged at 8000 × g for 20 min. The supernatants were used for the determination of glutathione peroxidase (GSH-Px, EC 1.11.1.9) and glutathione reductase (GSSGR, EC 1.6.4.2) activities. The Se dependent GSH-Px (H₂O₂ as substrate) and GSSGR were assayed according to the method of Hafeman et al.15 and to the method of Paglia and Valentine16 or the method of Whanger et al.,17 respectively.

Lipid peroxides in lung homogenates were determined as thiobarbituric acid reactive substances (TBARS). TBARS was performed according to the method of Masugi et al.18

The contents of GSH and GSSG in the lung were determined by the fluorometric method of Hissin and Hilf.19

Results
n-Hexanal and 4-HN in the Plasma, Liver, and Lung of Mice Injected the Authentic Aldehyde Figure 1 shows the time-dependent changes of the contents of n-hexanal or 4-HN in the plasma, liver, and lung following i.v. injection of each aldehyde. After the injection of n-hexanal, the plasma content reached ca. 500 nmol/ml at 5 min and decreased to less than half at 10 min. In the lung, the content of n-hexanal increased immediately and reached ca. 150 nmol/g in 30 min. From the mean of lung weight being ca. 0.17 g, the amount of n-hexanal in whole lung was ca. 26 nmol. This value corresponded to ca. 0.080% of the whole injected aldehyde. After 30 min, the content

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of n-hexanal decreased significantly. The amount of n-hexanal at 120 min was ca. 20% of that seen at 30 min. In the liver, the content of n-hexanal increased slightly 10 min after the injection. The content at 30 min decreased to the level of the untreated group.

In 4-HN, the plasma content of the aldehyde increased to ca. 320 nmol/ml at 5 min and decreased to less than half in 10 min. On the other hand, 4-HN in the lung reached ca. 520 nmol/g in 5 min. The amount corresponded to ca. 0.43% of the whole injected aldehyde. Forty percent of 4-HN observed at 5 min remained in the lung after 120 min. The excretion of 4-HN into the expired gas from the lung was slower than that of n-hexanal. In the liver, the content of 4-HN increased slightly 5 min after the injection in a similar manner as n-hexanal, but no accumulation of the 4-HN was observed.

TBARS and the Activities of Lipid Peroxide-Degradation Enzymes in the Lung of Mice Injected the Authentic Aldehyde TBARS in the lung tended to increase slightly by i.v. injection of n-hexanal or 4-HN, but no significant difference was observed between the control and the injected group (Table I). There is no change in the activities of GSH-Px and GSSGR by the injection of n-hexanal. Meanwhile, the activities of both enzymes decreased significantly by the injection of 4-HN. The GSH content in the lung decreased significantly by the injection of 4-HN, but no change in the contents of GSH and GSSG was observed by the injection of n-hexanal.

Discussion
Dillard and Tappel have indicated that n-hexanal produced in liver lipid peroxidation could be transferred into the lung. In this study, we found that 4-HN as well as n-hexanal were also transferred rapidly into the lung when these aldehydes were injected into the mouse tail vein. Especially, the accumulation of 4-HN in the lung increased to five times higher than that of n-hexanal 5 min after the injection and its content at 120 min was ca. 40% of that seen at 5 min. Though 4-HN content detected in the lung was very small in comparison with the injected amount, the amount would be enough to inhibit various enzymes. Actually, the accumulation of 4-HN resulted in lowering of the activities of GSH-Px and GSSGR and the contents of GSH in the lung. The decrease of GSH may be partly attributed to the decrease of GSSGR activities. When lipid peroxidation is usually generated, a decrease of GSH is attended by an increase of GSSG. But no increase of GSSG and TBARS in the lung was observed by the 4-HN treatment. Esterbauer has reported that 4-HN reacts with GSH, cysteine, and protein SH in in vitro experiment. The degradation effect of GSH in the lung was supposed to be caused by the binding of 4-HN to GSH without lipid peroxidation. The activity of superoxide dismutase (EC 1.15.1.1) in lung is higher than that of other organs such as liver. The ratios of vitamin E to polyunsaturated fatty acids in lung microsomes are about ten times higher than those of the liver. These reports have suggested that lungs which are directly exposed to the air would be more resistant to initiate lipid peroxidation than other organs and tissues. Therefore, it is thought that no significant lipid peroxidation was induced in spite of the decreases of antioxidative enzymes by 4-HN injection. However, it can be assumed that lipid peroxidation in the lung would be induced gradually after the period observed in this experiment since these enzymes and GSH play an important role against oxidative stress. n-Hexanal showed weak inhibitory effects on GSH-Px and GSSGR in comparison with those of 4-HN in in vitro study (data not shown). We observed no decrease of GSH content and GSH-Px and GSSGR activities in the lung by the injection of n-hexanal. Furthermore, n-hexanal content determined in the lung was lower than that of 4-HN. n-Hexanal but not 4-HN, is known to be readily metabolized by aldehyde metabolizing enzymes such as aldehyde dehydrogenase (EC 1.2.1.3). It is thought that n-hexanal could be either metabolized by the enzymes and/or transferred into the lung as such and excreted into expired gas easily, which is distinct from the 4-HN.

However, the degradation pathway of these aldehydes is complex and needs further investigation as to the relationships between the doses of aldehydes and their time-dependent effects on lipid peroxidation in lung.

References and Notes
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