THE EFFECT OF OZONE EXPOSURE IN VIVO ON THE APPEARANCE OF LUNG TISSUE LIPIDS IN THE ENDOBRONCHIAL LAVAGE OF RABBITS

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SUMMARY: Twenty hours after the injection of a mixture of [^{3}H] oleate and [^{14}C] palmitate lungs of rabbits exposed to 1 ppm ozone for 4 hours show a decreased incorporation of fatty acids (particularly [^{3}H] oleate) into lecithin. In addition the endobronchial lavage from these animals show increased specific activity of both [^{3}H] oleyl lecithins and [^{14}C] palmitoyl lecithins. These observations indicate that ozone may affect the lung by decreasing the formation of lecithins while simultaneouly stimulating release of surfactant lecithins.

INTRODUCTION: Recent evidence (1-3) indicates that lung tissue lipid including oleyl and palmitoyl lecithins (2-4) is the forerunner of alveolar wash lipid i.e. the extracellular component of alveolar surfactant (4). The specific activities of [^{3}H] oleyl lecithin and [^{14}C] palmitoyl lecithin in the lung tissue and alveolar washings are in equilibrium 20 hours after the injection of the radioactive fatty acid precursors (5). However there appears to be no studies on the factors affecting the secretion of alveolar surfactant. In an attempt to gain some information on the response of the lung to noxious gas (6) changes in the specific activities of [^{3}H] oleyl and [^{14}C] palmitoyl lecithins are used to investigate the rate of appearance of lecithins in endobronchial lavage when rabbits are exposed to ambient ozone.

MATERIALS AND METHODS: Male New Zealand white rabbits weighing 6-7 lbs, about 3 months old were fed ad libitum on standard rabbit chow for at least a week before being used. A mixture of 2.4 mCi [^{3}H] oleate and 0.3 mCi [^{14}C] palmitate (New England Nuclear, Boston Mass.) complexed with rabbit albumin (Sigma

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TABLE I

Incorporation of [^{3}H] Oleate and [^{14}C] Palmitate Into Lung Tissue and Alveolar Wash Lecithins by Control and Ozone Exposed Lungs

	cpm / μM Pi			
	[^{3}H] Oleate		[^{14}C] Palmitate	
	Lung Tissue	*Alveolar Wash*	*Lung Tissue*	*Alveolar Wash*
Control (20 hr after injection)	2750 ± 470	2200 ± 240	1690 ± 150	2180 ± 490
Exposed to ozone (from 16th to 20th hour)	1570 ± 370	2390 ± 720	1400 ± 320	2600 ± 250
Significant (P)	0.02	NS	NS	NS

Figures are means of six experiments (6 rabbits) ± SD.

Chemical Company, St. Louis, Mo.) at pH 7.4 were injected via the lateral auricular vein of each animal. Sixteen hours later the animals were divided into two groups of six. One group was exposed to 1 ppm of ozone for 4 hours, while the control group was allowed to breathe laboratory air for 4 hours. The animals were then sacrificed.

The cell-free endobronchial saline lavage containing alveolar surfactant was prepared as described by Young and Tierney (3). The lipids in the saline lavage, the residual or whole lung were extracted by the method of Folch et al. (7), with chloroform-methanol (2:1) containing 0.005 M butyrated hydroxy anisole, an anti-oxidant, fractionated by thin layer chromatography on plates coated with silica gel 60 HR (Brinkmann Instruments, Toronto) using the solvent system of Weinhold and Villee (8). The lecithin was eluted using 10 ml successive volumes of chloroform-methanol-acetic acid-water (100:60:16:8), chloroform-methanol (1:1) and methanol yielding recoveries of over 95%. All samples were dried and stored under N_2. Lecithin phosphorus was estimated by the method of Bartlett (9). Radioactivity was determined

TABLE 2

The ratio of the Specific Activity of Wash Lecithin and the Specific Activity of Tissue Lecithin

	Wash / Tissue Ratios	
	[^{3}H] Oleate	[^{14}C] Palmitate
Control	0.823 ± 0.205	1.303 ± 0.255
Exposed to Ozone	1.501 ± 0.130	1.815 ± 0.061
Significance (P)	0.01	0.05

Figures represent the means of 6 experiments ± SD.

in a Packard Tricarb scintillation counter model 3003, making appropriate corrections for spillage and quenching.

RESULTS: Twenty hours after the injection of the radioactive fatty acid lecithins from the lung tissue (residual lung) of animals exposed to 1 ppm of ozone for 4 hours showed a significant decrease in the specific activity of [^{3}H] oleyl lecithin compared to controls (Table 1). This difference is not reflected in the alveolar wash lecithins. Exposure to ozone did not produce a statistically significant difference in the specific activity of the [^{14}C] palmitoyl lecithin although a downward trend was consistently observed.

Table 2 compares the ratio of the specific activity of each radioactive fatty acid in wash lecithins and the tissue lecithins. It was noted that following ozone exposure this ratio increased significantly for the specific activities in both [^{3}H] oleyl and [^{14}C] palmitoyl lecithins.

The effect of ozone exposure on the molecular species of lung lecithins was also studied by comparing the [^{3}H] oleate/[^{14}C] palmitate ratio in the wash and

TABLE 3

$[^3H]$ Oleate / $[^{14}C]$ Palmitate Ratio in Lecithins

	Wash	Tissue
Control	1.011 ± 0.044	1.618 ± 0.139
Exposed to Ozone	0.919 ± 0.244	1.111 ± 0.305
Significance (P)	NS	0.05

Figures represent the means of 6 experiments ± SD.

tissue lecithins (Table 3). Although the molecular species were not specifically isolated since palmitate and oleate are incorporated predominantly into dipalmitoyl and 1-palmitoyl-2-oleyl lecithin (5) respectively, this ratio gives some indication as to the fate of the molecular species of lecithins following ozone exposure of rabbit lungs in vivo. The $^3H/^{14}C$ ratio in wash lecithins remains unchanged following ozone exposure. In the tissue however, the ratio on exposure decreased significantly compared to controls.

In addition ozone exposure increased ultraviolet absorption of lung lipids at 235 nm by 100% indicating lipid peroxidation, while hematocrit and erythrocyte osmotic fragility, indices of erythrocyte lysis and membrane damage; and lung weight body weight ratio an index of bronchial edema remained unchanged.

DISCUSSION: Saline lavage of lung yields material that meets the physical and chemical criteria for alveolar surfactant (3,8). In this study the supernatant at 1500 g x 5 mins was used as source of alveolar wash lipids. Although non-surfactant lipids are also present in the wash, Young and Tierney (3) have recently shown that following centrifugation at 1500 g x 5 mins there is no difference between the specific

activity of the lipids in the infranatant and supernatant fractions, and that 75% of the radioactive lipids are recovered in the supernatant. It is reasonable therefore, to assume that lipids of this fraction represent the extracellular compartment of alveolar surfactant.

Recently Roehm et al. (10) reported that ozone decreased rat lung linoleate and oleate, and that this effect was prevented by vitamin E (antioxidant) supplemented diet, thus implicating lipid peroxidation (11) as a mechanism by which ozone causes destruction of unsaturated lipids. Ozone is also known to attack sulfhydryl (-SH) containing compounds (12, 13, 14) a process which may interfere with acyl-CoA formation and its acylation in lipid biosynthesis. However, the decreased incorporation of [^{3}H] oleate into tissue lecithin cannot be explained exclusively by this mechanism since utilization of [^{14}C] palmitate is not significantly affected. It seems likely therefore that the decreased incorporation of [^{3}H] oleate into lung tissue lecithins demonstrated by the present study involves ozone interaction with the ethylenic linkage in oleate. The lack of a significant effect on the ratio of [^{3}H] oleate and [^{14}C] palmitate in alveolar wash lecithins may be a reflexion of the steady state nature of the alveolar surfactant pool(s) (10).

A comparison of the ratio of specific activities in the wash and tissue lecithins revealed that the rabbits exposed to ozone showed a significantly greater ratio than the controls, indicating increased specific activity of [^{3}H] oleyl- and [^{14}C] palmitoyl lecithins in alveolar wash lecithins following ozone exposure (Table 2). This observation is consistent with ozone induced increased release of a high specific activity pool of alveolar surfactant and with the existence in the lung tissue of a multipool intracellular surfactant system demonstrated recently by King and Clements (15). The increased ratio in the group exposed to ozone may be only partly due to decreased incorporation of radioactive fatty acids into tissue lecithins, because the [^{14}C] palmitate incorporation into lung lecithins is not significantly affected by ozone exposure (Table 1).

It has been reported that ozone exposure causes bronchial edema (15)

and alterations in the ultrastructure of alveolar tissue (16). Unlike the earlier studies (10,17) we used low levels of ozone (1 ppm) for a relatively short time (4 hours) and thus obtained no significant increase in lung weight/body weight ratios. It therefore seems unlikely that edema and erythrocyte damage play a significant role in the ozone induced appearance of surfactant in the endobronchial lavage indicated by our results. The possibility exists that the increased appearance in surfactant may be due to ozone induced stress (10) acting directly on the lung tissue via small airways and alveoli rather than an indirect one mediated by blood and lymph since hematocrit and osmotic fragility of erythrocyte remain unchanged.

Our results lead us to conclude that ozone may affect the lung by decreasing the formation and/or the amounts of existing lecithin while at the same time stimulating increased release of alveolar surfactant into the airways.

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