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EXPOSURE TO CRYSTALLINE SILICA OR TREATMENT WITH CHLORPHENTERMINE INCREASES VITAMIN E LEVELS IN RAT ALVEOLAR LAVAGE MATERIALS

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Previous studies have shown that vitamin E may be an integral part of lung surfactant and may function to protect this material from oxidant damage. Therefore, we measured the vitamin E levels in alveolar lavage materials from rats exposed to crystalline silica or treated with chlorphentermine (CP), two treatments that are known to increase surfactant phospholipids (PL) by different mechanisms. Silica exposure leads to increased PL synthesis, and CP treatment causes a reduction in PL degradation. Two different silica preparations, HCl-washed and unwashed silica, were used because exposure to each of them leads to different degrees of phospholipidosis. Exposure to HCl-washed silica results in a more than 17-fold increase in lavage PL and protein levels and a 12.2-fold increase in the amount of vitamin E. Exposure to unwashed silica leads to an approximately 7-fold increase in PL and proteins and a 5.8-fold increase in lavage vitamin E. Following treatment of rats with CP, there is a 15- to 19-fold increase in lavage PL and proteins and a 13.6-fold increase in vitamin E. When the results are expressed as micrograms vitamin E per milligram of lavage PL or protein, there is not much difference between controls and each treatment group. Because surfactant synthesis occurs in the endoplasmic reticulum, we also measured vitamin E in lung microsomes. Both silica exposure and CP treatment also lead to 1.8- to 2.5-fold increases, respectively, in the lung microsomal levels of vitamin E. These results demonstrate that alveolar lavage vitamin E levels are elevated along with lavage PL and proteins, and lung microsomal vitamin E levels are increased following exposure of rats to silica or treatment of the animals with CP.

Pulmonary surfactant is a mixture of phospholipids (PL) and proteins that lines the air–liquid interface in the lungs. Its major function is to reduce the surface tension at this interface and prevent alveolar collapse at low lung volumes. Due to its location on the alveolar surface, lung surfactant may be exposed to strongly oxidizing conditions

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and, thus, is susceptible to oxidant-induced alterations. This damage may be produced by exposure to gases, such as oxygen, nitrogen dioxide, or ozone, or as a consequence of increased release of reactive oxygen species (e.g., superoxide anion and hydrogen peroxide) and nitric oxide from alveolar macrophages and neutrophils during inflammatory reactions. Although the majority of surfactant phospholipids contain saturated fatty acids, there are significant amounts of unsaturated PL present (King & Clements, 1972; King, 1974). The unsaturated species may be particularly susceptible to oxidant damage. Seeger et al. (1985) have shown that exposure of lung surfactant to oxidative stress in vitro alters its surface tension reducing properties. Recently, Haddad et al. (1993) demonstrated that lung surfactant function is inhibited by in vitro treatment with peroxynitrite, a potent oxidizing agent formed from the combination of superoxide anion and nitric oxide, and the data suggest that lipid peroxidation of unsaturated PL may be involved. All of these results demonstrate that lung surfactant function may be altered by exposure to oxidants and that some of the damage may be due to oxidation of unsaturated PL.

One important protective mechanism for lung surfactant may be the presence of the lipid-soluble antioxidant vitamin E. It has been known for some time that supplementation of the diet with vitamin E has protective effects against some types of lung damage (Chow et al., 1984). Recently, Rüstow et al. (1993) reported that vitamin E is secreted as an integral part of pulmonary surfactant. In fact, their results suggest that the antioxidant becomes a part of surfactant by means of a specific process in type II cells, the major site of surfactant synthesis, and incorporation is not simply a result of the lipid solubility of the vitamin. Perhaps this packaging of vitamin E with surfactant occurs in the endoplasmic reticulum of type II cells, where surfactant lipid synthesis is known to occur (Wright & Clements, 1987).

Previous studies show that vitamin E may be an integral part of lung surfactant and may function to protect the material from oxidant damage. We are not aware of any studies designed to measure the surfactant vitamin E level if the surfactant phospholipid level is greatly increased by exposure of animals to particles or drugs. For example, it is well known that intratracheal instillations of crystalline silica in rats results in large increases in surfactant PL (Dethloff et al., 1986a; Miles et al., 1993). In this regard, we have shown that this response is greater when the silica is washed with HCl to remove iron contaminants than when unwashed native silica is used (Miles et al., 1994). This silica-induced phospholipidosis is due to an increase in the rate of surfactant PL synthesis (Miller & Hook, 1988; Dethloff et al., 1989). It has also been demonstrated that administration of chlorphentermine (CP), a cationic amphiphilic drug, to rats leads to increased amounts of surfactant PL (Reasor & Heyneman, 1983; Miles

et al., 1986). The CP-induced response appears to be due to a reduction in the rate of PL degradation (Miles et al., 1986). Both the silica- and CP-induced conditions would presumably put the lungs at an increased risk for oxidant-mediated damage unless antioxidant levels increase correspondingly. Kacew and Narbaitz (1980, 1981) reported that hyperoxia increased the phospholipogenic response to CP in newborn rats as evidenced by the enhanced development of alveolar foam cells. In other studies, hyperoxia altered biochemical properties in lungs of CP-treated newborn rats (Kacew et al., 1981a), although the toxicological significance of these changes is uncertain. Also, Gairola et al. (1983) demonstrated that CP-treated rats receiving a vitamin E-deficient diet had higher levels of lipid peroxidation and alveolar foam cell disintegration than drug-treated rats receiving vitamin E supplemental diets. Furthermore, rats receiving a vitamin E-deficient diet and receiving CP have structural alterations in alveolar type II cells (Matulionis et al., 1983).

The objective of our current investigation was to study the relationship between surfactant-associated vitamin E and phospholipids in alveolar lavage materials obtained from untreated rats and animals treated with HCl-washed crystalline silica, unwashed silica, or chlorphentermine. Because surfactant phospholipid synthesis, and perhaps vitamin E packaging with the lipids, is known to occur in endoplasmic reticulum, we also measured the vitamin E levels in the lung microsomal fractions.

METHODS

Treatment of Animals with Silica or Chlorphentermine

Male Sprague-Dawley rats (CVF) (225–250 g) obtained from Hilltop Laboratories (Scottdale, PA) were used in all experiments. Rats were housed six to a cage in suspended wire cages. In one series of experiments, the rats were exposed to crystalline silica (Min-U-Sil; <5 μm in diameter) that was obtained from the Pennsylvania Glass Sand Corporation (Pittsburgh, PA). Animals were exposed to silica washed with HCl to remove contaminating Fe_2O_3 (Dethloff et al., 1986b) or to the unwashed material. HCl-washed silica was prepared by boiling the particles in 1.0 *M* HCl, washing 4 times with water, and drying in an oven at 110°C (Dethloff et al., 1986b; Miles et al., 1993). Unwashed silica particles were washed 4 times with water and dried in an oven at 110°C. Both the HCl-washed and unwashed silica particles were then kept in an oven at 200°C overnight to sterilize them. Sterilized particles were suspended in sterile saline and sonicated immediately prior to the intratracheal instillations. Treated animals received a single dose of 20 mg of either washed or unwashed silica,

while control animals received saline alone. For intratracheal instillations, the rats were lightly anesthetized with methohexital sodium (35 mg/kg body weight) and placed in a vertical position. A curved cannula (18 gauge) was then inserted into the trachea and positioned just above the bronchial bifurcation. After administration of the particles or saline, the animals were returned to their cages and sacrificed 2 wk later. The dose of silica (20 mg) and the time period following exposure (2 wk) were used because we have already established that this treatment regimen produces significant phospholipidosis (Miles et al., 1994). In another series of experiments, the rats were treated with CP. The animals were given intraperitoneal injections of chlorphentermine hydrochloride dissolved in saline (25 mg/kg body weight, PreSate, Warner-Chilcott, Morris Plains, NJ). The control group of animals was injected with an equal volume of saline alone. Treatment consisted of 10 injections given over a 2-wk period (no weekend treatment). This treatment regimen also induces a significant phospholipidosis (Reasor & Heyneman, 1983). The control group was food restricted so that its weight gain (20 ± 3% of the initial body weight) was not significantly different compared to the CP group (weight gain of 16 ± 3% of initial body weight).

Preparation of Alveolar Lavage Materials and Lung Microsomes

Following the appropriate treatment period, the animals were anesthetized with sodium pentobarbital (150 mg/kg body weight), exsanguinated by cutting the abdominal aorta, and the heart and lungs were removed. The lungs were perfused with 0.9% NaCl to remove blood. The heart and connective tissue were then removed, and the trachea and lungs were blotted and weighed. Alveolar lavage materials were obtained by tracheal lavage (3 lavages; 9 ml each) with a Tris maleate-buffered medium (150 m*M* NaCl, 5 m*M* Tris maleate, pH 7.4), a medium that is used in the measurement of vitamin E levels (Taylor et al., 1976; Kornbrust & Mavis, 1980). Ethylenediamine tetraacetic acid (EDTA) was added to the lavage materials immediately after their collection so that the final concentration was 1 m*M*. Alveolar cells were removed from the lavage fluid by centrifugation at 300 × g for 5 min. The cells were washed 3 times and all washings were spun at 15,000 × g for 10 min. The materials obtained from these washings were included with alveolar lavage materials because a significant amount of rat lavage phospholipids is recovered from this pellet (Miles et al., 1986). All cell-free alveolar lavage materials were then spun at 100,000 × g for 2 h to obtain a pellet. This pellet was then resuspended in the Tris maleate buffer (with EDTA) and stored at 2°C prior to analysis. The lavage materials were flushed with argon and sealed following each step of preparation in order to preserve the vitamin E.

After the lavages were performed, lung microsomes were obtained according to the method of Miles et al. (1993). Briefly, the lungs were trimmed free of the trachea, bronchi, and connective tissue, and the tissue was minced by chopping four times with a tissue chopper (Mickle Engineering Co., Gomshall, Surrey, UK) set at a slice thickness of 0.5 mm. The minced tissue was resuspended in ice-cold Tris maleate-buffered medium (with 1 m*M* EDTA) maintained at 2°C, and a 25% (w/v) tissue homogenate was prepared by using a Teflon–glass Potter–Elvejhem homogenizer. A microsomal pellet was obtained by differential centrifugation, resuspended in Tris maleate-buffered medium (with EDTA), and stored on ice until used for measurements. The protein content was measured (Lowry et al., 1951), and the microsomes were shown to be free of mitochondrial contamination (Singer & Kearney, 1957). These samples were also flushed with argon and sealed following each step of preparation.

Measurement of Phospholipid, Protein, and Vitamin E Levels

The phospholipid (PL), protein, and vitamin E levels in the 100,000 × g pellet from alveolar lavage materials and the protein and vitamin E contents of lung microsomes were measured. Phospholipids in the lavage materials were measured as the phosphorus present in lipid extracts (Miles et al., 1986; Bartlett, 1959). The PL content was determined by multiplying lipid phosphorus values by 25 (Oyarzun & Clements, 1978). Protein in the lavage was measured by the method of Lowry et al. (1951) with 1% sodium dodecyl sulfate added to reduce interference by lipids (Lees & Paxman, 1972). Because the lavage returns were similar in each series of experiments, and varied only between the extremes of 90(±2)% and 93(±1)%, the results were expressed as milligrams of phospholipid or protein in the entire lavage pellet.

Vitamin E in the alveolar lavage pellets and lung microsomal fractions was measured with a fluorometric technique described by Taylor et al. (1976). Briefly, samples of lavage materials or microsomes were diluted with Tris maleate-buffered medium (with EDTA) so that the final volume was 1.5 ml. Saponification mixtures consisting of the sample (1.5 ml), 0.5 ml of 25% ascorbic acid, and 1.0 ml absolute ethanol were preincubated at 70°C for 5 min. Following the addition of 10 *N* KOH (1 ml), the mixtures were saponified for 30 min at 70°C. The mixtures were then cooled and extracted with 4 ml hexane by vortexing for 1 min. Then the extracted mixtures were centrifuged at 500 × g for 5 min, and the hexane phase was removed. Fluorometric measurement of vitamin E in the hexane phase was carried out at an excitation wavelength of 286 nm and an emission wavelength of 330 nm. Standard curves were constructed from samples of α-tocopherol, which were taken through the entire saponifica-

tion and extraction procedure. All measurements were made with a model LS-50 luminescence spectrophotometer (Perkin-Elmer Corp., Norwalk, CT). We determined there was no vitamin A interference in our measurements by finding no differences in fluorescence of the samples after addition of H_2SO_4 (Taylor et al., 1976). Our results were expressed as micrograms of vitamin E in the samples.

Statistics

For the experiments in which animals were exposed to silica, the comparisons among control and treatment groups were made using analysis of variance (ANOVA). Tukey's protected *t*-test was used to test the significance of differences between groups of animals. For the experiments in which animals were treated with CP, comparisons of statistical significance between control and treated groups were made by using the unpaired Student's *t*-test. The criterion used for significance in all comparisons was $p < .05$.

RESULTS

Intratracheal Exposures to Crystalline Silica

Following exposures of rats to either HCl-washed or unwashed silica, there were no significant changes in body weights (Table 1). However, there were 65% and 25% increases in lung weights following treatment with the washed and unwashed silica, respectively. The PL, protein, and vitamin E contents of the 100,000 × g alveolar lavage pellets are also shown in Table 1. Both the PL and protein contents are

TABLE 1. Body and lung weights and alveolar lavage phospholipid (PL), protein, and vitamin E levels in control and crystalline silica-exposed rats

	Treatment		
Parameter (units)	Control	HCl-washed silica	Unwashed silica
Body weight (g)	289 (±3)	287 (±5)	280 (±3)
Lung weight (g)	1.74 (±0.04)	2.88 (±0.11)[a]	2.16 (±0.07)[b]
PL (mg)	2.00 (±0.09)	34.59 (±1.07)[a]	13.38 (±0.39)[b]
Protein (mg)	0.80 (±0.05)	14.01 (±1.12)[a]	5.65 (±0.47)[b]
Vitamin E (μg)	1.74 (±0.08)	21.30 (±1.14)[a]	10.03 (±0.42)[b]
μg Vitamin E/mg PL	0.88 (±0.07)	0.62 (±0.04)[a]	0.75 (±0.03)
μg Vitamin E/mg protein	2.22 (±0.16)	1.51 (±0.10)[a]	1.81 (±0.15)

Note. Values shown are means ± SEM for data from five to eight animals in each treatment group.

[a,b]For a given parameter, groups with different letter superscripts are significantly different from each other and from groups without superscripts ($p < .05$).

TABLE 2. Vitamin E and protein contents of lung microsomal fractions from control and crystalline silica-exposed rats

Parameter (units)	Treatment		
	Control	HCl-washed silica	Unwashed silica
Protein (mg)	3.53 (±0.10)	8.53 (±0.25)[a]	6.33 (±0.18)[b]
Vitamin E (μg)	2.89 (±0.20)	7.07 (±0.33)[a]	5.97 (±0.29)[b]
μg vitamin E/mg protein	0.82 (±0.06)	0.83 (±0.05)	0.91 (±0.05)

Note. Values shown are means ± SEM for data from five to eight animals in each treatment group.

[a,b]For a given parameter, groups with different letter superscripts are significantly different from control and from each other ($p < .05$).

increased more by exposure to HCl-washed than to unwashed silica; that is, the PL is increased by 17.3- or 6.7-fold, and protein is increased by 17.5- or 7-fold following treatment with HCl-washed or unwashed silica, respectively. These results confirm our previous findings (Miles et al., 1994). The effects of these two particles on vitamin E levels in the lavage pellets are qualitatively similar to their effects on PL and proteins. Exposure to washed silica leads to a 12.2-fold increase, while treatment with the unwashed material results in only a 5.8-fold increase in vitamin E. When the results are expressed as micrograms of vitamin E per milligram of PL or protein, there are no significant differences between control and rats treated with unwashed crystalline silica. However, exposure to acid-washed silica leads to a decrease in the ratios of vitamin E to PL and to protein. These results show that exposure of rats to silica leads to an increase in alveolar lavage vitamin E levels and that these increases correspond, in general, to the enhanced production of PL and proteins.

We measured the protein and vitamin E levels in lung microsomes, and the results are shown in Table 2. As we reported previously (Miles et al., 1993, 1994), exposure of rats to silica leads to an increase in the amount of microsomal protein that can be obtained from the lungs, and the increase is more pronounced following treatment with HCl-washed (2.4-fold increase) than with exposure to unwashed silica (1.8-fold increase). Vitamin E levels are also increased to approximately the same extent. In fact, when the data are expressed as micrograms vitamin E per milligram microsomal protein, there are no differences between control and either treatment group. These results show that exposure of rats to silica leads to increases in the vitamin E levels in lung microsomes, and that the increase is approximately the same as the increase in microsomal protein.

Administration of Chlorphentermine

The effects of CP treatment on body and lung weights and alveolar lavage pellet PL, protein, and vitamin E levels are shown in Table 3. There was no significant difference in the body weights of CP-treated and control rats. However, drug treatment leads to increases of 1.3-fold in lung weight, 18.7-fold in PL content, and 15-fold in protein levels. These results are similar to those we reported previously (Miles et al., 1986). CP treatment also produces a 13.6-fold increase in lavage vitamin E content. When the data are expressed as micrograms vitamin E per milligram of either phospholipid or protein, there are no significant differences between CP-treated and control animals. These results show that the alveolar lavage vitamin E content is elevated following treatment of rats with CP and that this increase is quantitatively similar to the increases in lavage PL and protein.

The effects of drug treatment on lung microsomal protein and vitamin E levels are shown in Table 4. Administration of CP leads to a 1.8-fold increase in microsomal protein content. However, the drug effect on microsomal vitamin E content is greater, that is, a 2.5-fold increase. It follows then that when the data are expressed as micrograms vitamin E per milligram microsomal protein, the ratio is greater in microsomes from CP-treated animals.

DISCUSSION

In this study, we demonstrated that the alveolar lavage vitamin E levels are increased when the surfactant phospholipid level is elevated either by an increase in its rate of synthesis (intratracheal exposure of rats to silica) or by a decrease in its rate of degradation (treatment of

TABLE 3. Body and lung weights and alveolar lavage phospholipid (PL), protein, and vitamin E levels in control and chlorphentermine (CP) treated rats

	Treatment	
Parameter (units)	Control	CP
Body weight (g)	273 (±7)	271 (±6)
Lung weight (g)	1.78 (±0.05)	2.33 (±0.07)[a]
PL (mg)	1.65 (±0.11)	30.91 (±3.50)[a]
Protein (mg)	0.74 (±0.03)	11.11 (±1.60)[a]
Vitamin E (µg)	2.77 (±0.33)	37.58 (±2.00)[a]
µg Vitamin E/mg PL	1.56 (±0.10)	1.32 (±0.19)
µg Vitamin E/mg protein	3.75 (±0.45)	3.73 (±0.50)

Note. Values shown are means ± SEM for data from five to six animals in each treatment group.

[a]Significant difference ($p < .05$) from the corresponding value for control animals.

TABLE 4. Vitamin E and protein contents of lung microsomal fractions from control and chlorphentermine (CP) treated rats

	Treatment	
Parameter (units)	Control	CP
Protein (mg)	5.12 (±0.47)	9.20 (±0.40)[a]
Vitamin E (μg)	3.47 (±0.30)	8.68 (±0.20)[a]
μg Vitamin E/mg protein	0.70 (±0.08)	0.95 (±0.02)[a]

Note. Values shown are means ± SEM for data from five to six animals in each treatment group.

[a]Significant difference ($p < .05$) from the corresponding value for control animals.

animals with chlorphentermine, CP). In each case, the amount of vitamin E is increased to roughly the same extent as the lavage phospholipids or proteins. In addition, exposure of rats to silica or treatment of the animals with CP leads to greater vitamin E and protein levels in lung microsomes. Furthermore, the amount of vitamin E is increased to approximately the same extent as the microsomal protein levels by each treatment.

It is well known that intratracheal exposures of rats to silica lead to increased rates of surfactant phospholipid synthesis (Miller & Hook, 1988; Dethloff et al., 1989). In these experiments, we used HCl-washed and unwashed silica preparations to increase surfactant PL synthesis to different degrees (Miles et al., 1994). It is interesting to note that the increases in alveolar lavage PL levels are the same as the increases in protein levels. For example, exposure to acid-washed silica preparations results in approximately 17- to 18-fold elevations in lavage PL and protein levels, while exposure to unwashed silica leads to 7-fold increases in these 2 lavage components. The alveolar lavage vitamin E content is also elevated to different degrees following the exposure of rats to the two silica preparations. As is the case with lavage PL and proteins, alveolar vitamin E is elevated more by acid-washed silica treatment than by exposure to the unwashed material. In fact, the ratios of either vitamin E to PL or vitamin E to protein are not different in alveolar lavage materials from those in control and unwashed silica-treated animals. On the other hand, both of these ratios are slightly reduced in lavage materials from rats exposed to HCl-washed silica relative to those from controls and unwashed silica-treated rats.

Treatment of rats with chlorphentermine is known to result in large increases in alveolar lavage phospholipids (Reasor & Heyneman, 1983; Miles et al., 1986), presumably due to a reduced rate of PL degradation (Miles et al., 1986). The results of our current study demonstrate

that CP treatment leads to large increases in alveolar lavage PL, proteins, and vitamin E. There are 15- to 18-fold increases in PL and protein levels, while lavage vitamin E is elevated by almost 14-fold. The ratios of vitamin E to either PL or protein are not different in lavage materials from control and CP-treated animals.

The mechanism(s) by which alveolar vitamin E levels are elevated following exposure to silica or treatment with CP is not known. In the case of silica exposures, one possibility is that vitamin E is elevated due to the increased rate of surfactant synthesis. Rüstow et al. (1993) suggested that vitamin E is packaged and secreted with surfactant as an integral part of that material, at least in untreated animals. In this regard, it is possible that the ratios of alveolar vitamin E to PL or proteins are slightly reduced in rats exposed to HCl-washed silica because the rate of surfactant synthesis is increased to such an extent that vitamin E levels do not keep up. On the other hand, the CP-induced elevation in the amounts of surfactant materials is thought to be due to decreased rates of degradation and not to increased rates of synthesis. In this case, why has the lavage vitamin E not been oxidized? One possibility is that the vitamin E is re-reduced after its oxidation and before its decomposition. Two substances that are known to regenerate vitamin E are the water-soluble antioxidants glutathione and ascorbic acid (Packer & Landvik, 1989). In this regard both glutathione and ascorbate have been found in alveolar lavage materials (Cantin et al., 1987; Snyder et al., 1983). Also, Kacew et al. (1981a) reported that treatment with CP increased activities of glutathione-related enzymes in newborn rat lungs, and Reasor and Koshut (1980) found increases in these enzyme activities in alveolar macrophages of CP-treated rats. On the other hand, there may be a very simple explanation for both the silica- and CP-induced increases in alveolar lavage vitamin E levels, that is, the lipid solubility of the antioxidant. In each case, the elevations in the alveolar lipid levels may lead to increases in the lipid-soluble vitamin E levels, perhaps derived from an extracellular source, such as plasma.

Both exposure to silica and treatment with CP lead to increased levels of lung microsomal protein. The silica-induced response has been reported previously (Miles et al., 1993). In contrast, Kacew et al. (1981b) reported that CP treatment did not increase microsomal protein in newborn rat lungs. There are also large increases in lung microsomal vitamin E in both silica- and CP-treated rats. In this regard, it is known that the content of vitamin E in lung microsomes is among the highest of microsomal tissue from all organs of untreated rats (Kornbrust & Mavis, 1980). Although the reason(s) for the silica- and CP-induced increases of the already high lung microsomal vitamin E levels are not known, there is at least one possibility. It is well known that lung vitamin E levels are adaptively increased following

insults such as cigarette smoking (Chow et al., 1989) and exposure to NO_2 (Elsayed & Mustafa, 1982) or ozone (Elsayed, 1982). Accordingly, it is well known that exposure to silica leads to inflammation (Vallyathan et al., 1988) and treatment with CP leads to conditions that favor lipid peroxidation in the lungs (Reasor & Koshut, 1980). Perhaps these oxidizing conditions are involved in the elevation of lung microsomal vitamin E levels. It is also possible that there is more vitamin E secreted with lung surfactant PL in these treated animals, since the endoplasmic reticulum is the site of surfactant synthesis (Wright & Clements, 1987).

There is one peculiar finding in the results of these experiments. The amount of vitamin E in alveolar lavage materials from control rats used in the CP experiments is 60% greater than that from control rats used in the silica experiments, although the lung weights and lavage PL and protein levels are not different. The reason(s) for this difference is not known. The animals used for these two groups of experiments were obtained 3 mo apart, but their diets, both that used by the supplier and that used in our animal facility, did not differ other than that the CP controls were food restricted and the silica controls were not. The differences in control vitamin E levels should not affect the results of our experiments, however, since we are only interested in relative changes caused by each treatment. Furthermore, the changes in alveolar lavage PL, protein, and vitamin E levels resulting from each treatment are far greater than the differences between the two sets of controls.

In summary, exposure of rats to silica or treatment of the animals with CP leads to increases in the amounts of alveolar lavage vitamin E that are approximately proportional to the increases in the levels of lavage phospholipids and proteins. Although the lung microsomal levels of vitamin E are also increased by these treatments, the mechanism by which the vitamin E in alveolar lavage materials is elevated is not known. Both exposure to silica and treatment with CP may put lung surfactant at risk for oxidative damage. For example, it is well known that exposure to silica may result in the production of free radicals (Vallyathan et al., 1988) and that conditions that favor lipid peroxidation exist in the lungs of CP-treated rats (Reasor & Koshut, 1980). Thus, the higher vitamin E levels in alveolar lavage materials from these treated animals may play an important protective role.

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