TAURINE PROTECTS AGAINST OXIDANT INJURY TO RAT ALVEOLAR PNEUMOCYTES

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INTRODUCTION

Taurine (2-aminoethanesulfonic acid) is an unusual amino acid found in a wide variety of animal species. Its precise role in human and animal nutrition has remained unclear despite intensive investigation. Recently, however, evidence has accumulated supporting the hypothesis that taurine protects cellular membranes against toxic compounds, including bile acids, xenobiotics, and oxidants. In different experimental systems, taurine has been demonstrated to act as a direct (primary) antioxidant that scavenges oxygen-free radicals and as an indirect (secondary) antioxidant that prevents changes in ion transport and membrane permeability which result from oxidant injury[1-3].

Exposure to oxidant gases, including hyperbaric oxygen, nitrogen dioxide, and ozone, results in pulmonary injury[4-7]. The morphological changes which occur in lung tissue include destruction of capillary endothelial cells, edema, hypertrophy and hyperplasia of the bronchiolar epithelium, bronchiolization of the alveolar duct epithelium, and an influx of macrophages and polymorphonuclear leukocytes into the alveolar air spaces. Metabolic changes which result from oxidant injury to the lung include lipid peroxidation and mobilization of cellular antioxidants, such as glutathione, ascorbic acid, and vitamin E.

Although hyperoxia and NO_2 or ozone exposures result in similar morphological

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and metabolic changes in the lung, the distribution of the injury varies; NO_2 and ozone toxicities primarily involve the epithelium of the conducting airways and alveoli near the terminal bronchioles (proximal alveoli), whereas exposure to high oxygen tensions induces damage at the levels of the trachea through the distal alveoli. Dietary taurine supplementation has previously been shown to protect against bronchiolar damage induced by the oxidant gas NO_2[8].

Pneumocytes found on the alveolar surface exhibit a wide range of sensitivities to oxidant injury. Alveolar type II epithelial cells are relatively resistant to oxidant injury while type I cells are the most susceptible to it. In fact, type II cells are thought to repair oxidant induced damage to the alveolar epithelium by replacing injured type I cells. Alveolar macrophages are relatively insensitive to damage by oxidant gases *in vivo*[9]; however, *in vitro*, they appear to be more sensitive than type II cells to oxidant injury[10].

The studies described here were conducted to investigate the possible role of taurine as an antioxidant in alveolar macrophages.

METHODS

Isolation of Lung Cells and Exposure to Ozone

Rats (male Sprague Dawley) weighing 200-300 g were anesthetized with pentobarbital sodium (65 mg/kg body wt). Alveolar macrophages were obtained by pulmonary lavage, as previously described[11-13]. Cell yield for fast preparation was determined with a Coulter electronic cell counter. Mean cell volumes and the purity of each preparation were determined using an electronic cell sizing attachment. The cellular preparations averaged 91 $\pm$ 1% alveolar macrophages.

Isolated alveolar macrophages were allowed to adhere to the bottom surface of a 75-cm^2 tissue culture flask. The flask was mounted on an orbital mixer, and the cells were exposed to 0.45 $\pm$ 0.05 ppm ozone for 60 min at room temperature while the flask was slowly rocked from side-to-side (22 cycles/min) to allow direct contact between the cells and the oxidant gas. After ozone exposure, the medium was recovered and the non-adherent and adherent cell fractions were combined prior to conducting assays on the medium and the cells[12,13].

Measurement of Lung, Plasma, Extracellular and Intracellular Taurine Levels

Taurine was isolated from tissue, cells and media in the experiments using dual-bed ion-exchange chromatography[14], and taurine contents were measured by an HPLC technique which we developed[15]. This method involves deproteinized samples and therefore measures free (i.e. not protein-bound) taurine, rather than total taurine.

Cell and Medium Assays

To measure cell viability, the exclusion of trypan blue dye was determined microscopically as previously described[12,13]. Chemiluminescence (both resting and symosan-stimulated) was measured as previously described[12,13], using luminol and a Packard Tri-Carb scintillation counter operated in the out-of-coincidence mode. Membrane ATPase activities were measured as inorganic phosphate liberated from ATP, as previously described[12,13]. To estimate the activity of Na^+/K^+ ATPase by difference, ouabain was added to half of the samples.

Lipid peroxides released into the medium were measured spectrophotometrically as thiobarbituric acid-reactive substances, by the method previously described. Protein leakage into the medium was estimated with the Bio-Rad technique as previously described. Leakage of reduced and oxidized glutathione into the medium was

determined fluorometrically, by the method previously described. Potassium ion leakage was measured by atomic absorption spectroscopy, as previously described[12,13].

Statistical Analyses

Data reported are expressed as means ± standard errors of at least three separate experiments using cells or tissues pooled from groups of rats euthanized on at least 3 separate days. Statistical differences between data points were estimated using either the Student's t test or one-way ANOVA. The significance level was set at $p < 0.05$.

RESULTS

Effects of In Vitro Ozone Exposure on Peroxidative Damage, Membrane Leakage, and Taurine Content of Rat Alveolar Macrophages

Recovery of macrophages from the culture flask averaged 57.7% with cell populations that were not exposed to ozone. Recovery was not affected after a 15-min exposure to ozone but decreased significantly after 30 min of ozone exposure. Initially, adherent cells represented 60% of the recoverable macrophages. The ratio of adherent to non-adherent macrophages remained relatively constant for the first 30 min of ozone exposure. However, after a 60-min treatment with ozone, only 44% of recoverable cells were adherent. Similarly, cell viability, as estimated by trypan blue exclusion, was maintained for the first 30 min of ozone exposure and declined thereafter (Figure 1). The decreasing yield of macrophages from the culture flask

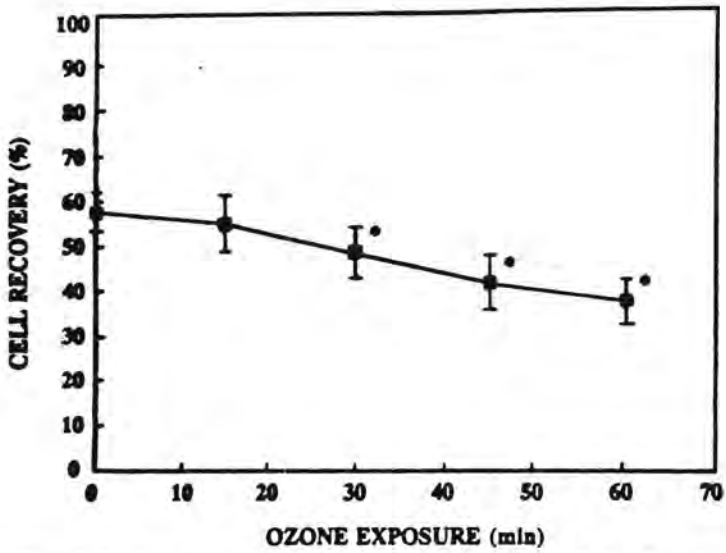

Figure 1. Viability of alveolar macrophages recovered from tissue culture flasks after exposure to ozone for 0-60 min (mean ± SEM for N = 5-7 observations). *A significant decrease from the value at 0 min at the $p < 0.05$ level (*t* test).

with increasing ozone exposure may reflect decreasing cell viability and/or changes in the adherent properties of the cells.

Cellular chemiluminescence, which is a reflection of free radical generation (including reactive oxygen and lipid metabolites), is given in Table 1. Initially, ozone exposure caused increased chemiluminescence in both resting and zymosan-stimulated macrophages. This response peaked at 15-30 min of exposure and declined to the basal level by 60 min of exposure to ozone. At 15 through 45 min of exposure, zymosan-stimulated chemiluminescence greatly exceeded resting values, indicating that the ability of particles to enhance the production of reactive oxygen metabolites is maintained in ozone-exposed macrophages.

Total membrane ATPase activity decreased with increasing length of ozone exposure (Table 2) and reached a maximum at 30 min of exposure. The Na^+/K^+ ATPase activity (which is ouabain-sensitive) also decreased with time and was undetectable by 30 min of exposure.

Lipid peroxides were detectable in the extracellular medium. Lipid peroxidation products were significantly elevated after 15 min of exposure to ozone and their production increased linearly with increasing exposure time (Figure 2).

Protein leakage from cultured alveolar macrophages also linearly increased with increasing length of ozone exposure (Figure 3). However, protein leakage was not significantly elevated until 30 min of exposure.

Leakage of reduced glutathione into the medium (Figure 4) increased significantly after 30 min of exposure to ozone and significantly declined thereafter. Leakage of oxidized glutathione into the medium (Figure 4) increased by 15 min and remained constant from 15-60 min of ozone exposure.

Table 1. Effect of ozone exposure on chemiluminescence in rat alveolar macrophages[a].

Ozone exposure time (min)	Chemiluminescence[b]			
	Resting[c]	Zymosan-stimulated[d]	Difference[e]	Ratio[f]
0	0.036 ± 0.002	0.121 ± 0.007	0.084 ± 0.006	3.3 ± 0.1
15	0.266 ± 0.017 (7.4)[g]	1.639 ± 0.032 (13.5)[g]	1.373 ± 0.024[g]	6.2 ± 0.3[g]
30	0.293 ± 0.006 (8.1)[g]	1.404 ± 0.176 (11.6)[g]	1.226 ± 0.137[g]	4.8 ± 0.5[g]
45	0.178 ± 0.027 (4.9)[g]	0.882 ± 0.186 (7.3)[g]	0.705 ± 0.179[g]	4.9 ± 0.9[g]
60	0.093 ± 0.005 (2.6)	0.147 ± 0.007 (1.2)	0.054 ± 0.007	1.6 ± 0.1

[a] Values represent means ± SEM for three observations.
[b] Relative chemiluminescence determined gravimetrically as the area under the curve of cpm vs time.
[c] Activity in the absence of 2 mg/ml zymosan.
[d] Activity in the presence of 2 mg/ml zymosan.
[e] Zymosan-stimulated minus resting values.
[f] Zymosan-stimulated values divided by resting values.
[g] Increase relative to value at 0 min.

The intracellular taurine concentration increased with increasing length of exposure to ozone, peaked at 30 min, and then declined (Figure 5). Extracellular taurine levels increased with increasing length of ozone exposure (Figure 6).

Potassium ion leakage increased with increasing length of exposure to ozone (Figure 7). The increase in extracellular K^+ concentration can be correlated with the decrease in activity of the Na^+/K^+ ATPase from 0 to 30 min of ozone exposure (Table 2).

Table 2. Effect of ozone exposure on membrane ATPase activity of rat alveolar macrophages[a]

Ozone exposure time (min)	Total ATPase[b] (nmol PO_4/hr/10^6 cells)	Na^+/K^+ ATPase[c] (nmol PO_4/hr/10^6 cells)
0	50.7 ± 2.1	45.2 ± 2.3 (89%)[d]
15	31.8 ± 2.0*	16.4 ± 1.8 (52%)*
30	10.0 ± 3.0*	−1.5 ± 3.7 (~0)*
45	9.9 ± 0.7*	−3.1 ± 2.8 (~0)*
60	9.9 ± 0.8*	−2.6 ± 1.6 (~0)*

[a] Values represent means ± SEM for three observations.

[b] Activity in the absence of 1 mM ouabain.

[c] Difference between total activity and activity in the presence of 1 mM ouabain.

[d] Percentage of total ATPase activity.

* A significant decrease from the value at 0 min at the $p < 0.05$ level (t test).

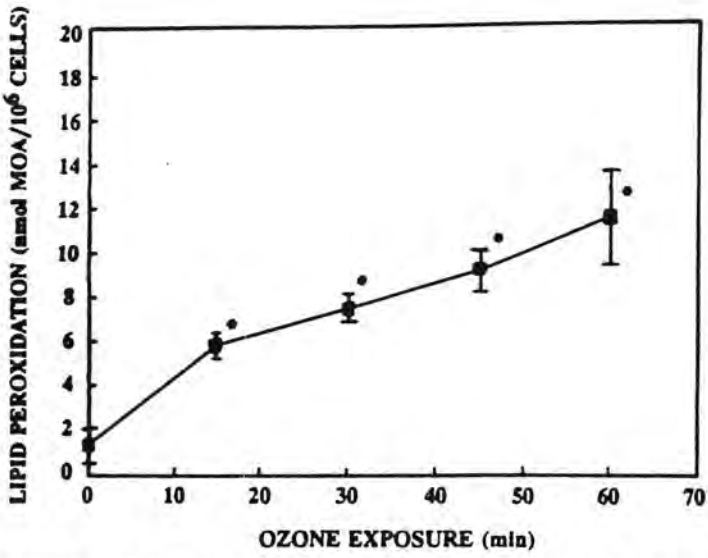

Figure 2. Appearance of lipid peroxidation products in the medium after exposure of alveolar macrophages to ozone for 0-60 min (mean ± SEM for N = 5-7 observations). *A significant increase from the value at 0 min in the $p < 0.05$ level (t test).

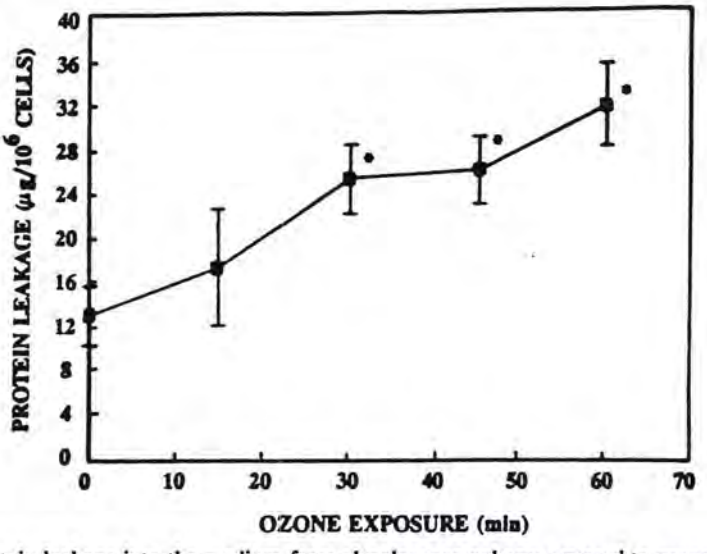

Figure 3. Protein leakage into the medium from alveolar macrophages exposed to ozone for 0-60 min (mean ± SEM for N = 4-6 observations). *A significant increase from the value at 0 min at the $p < 0.05$ level (*t* test).

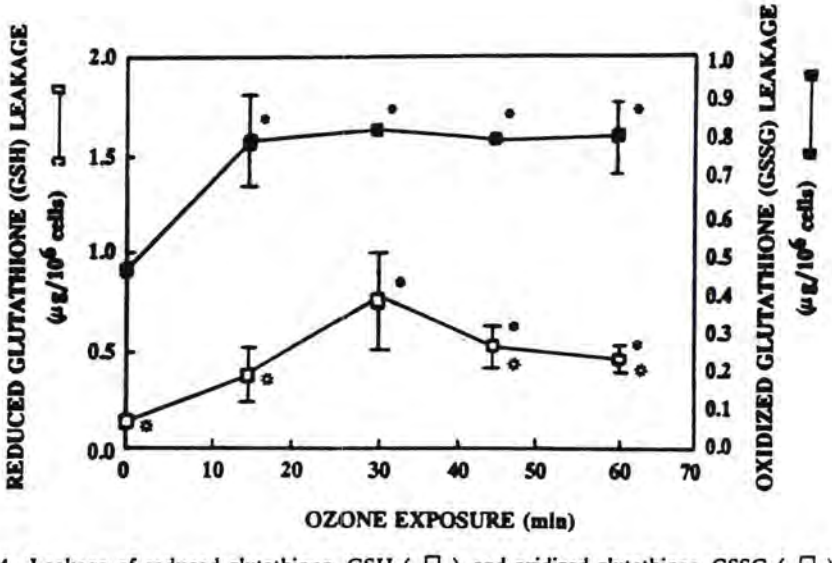

Figure 4. Leakage of reduced glutathione, GSH (□), and oxidized glutathione, GSSG (□), from alveolar macrophages exposed to ozone for 0-60 min (mean ± SEM for N = 3-7 observations). *A significant increase from the value at 0 min at the $p < 0.05$ levels (*t* test). ✡ A significant decrease from the value at 30 min at the $p < 0.05$ level (*t* test).

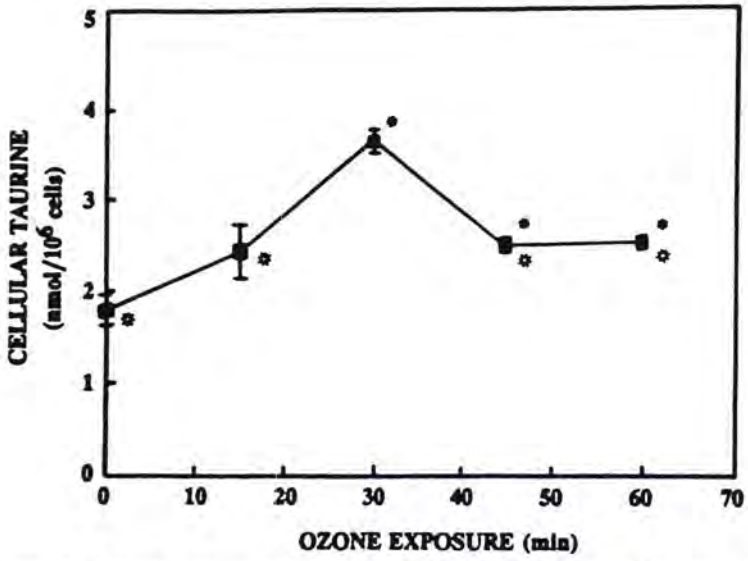

Figure 5. Intracellular taurine concentrations in alveolar macrophages exposed to ozone for 0-60 min (mean ± SEM for N = 3 observations). *A significant increase from the value at 0 min at the $p < 0.05$ level (*t* test). ¤ A significant decrease from the value at 30 min at the $p < 0.05$ level (*t* test).

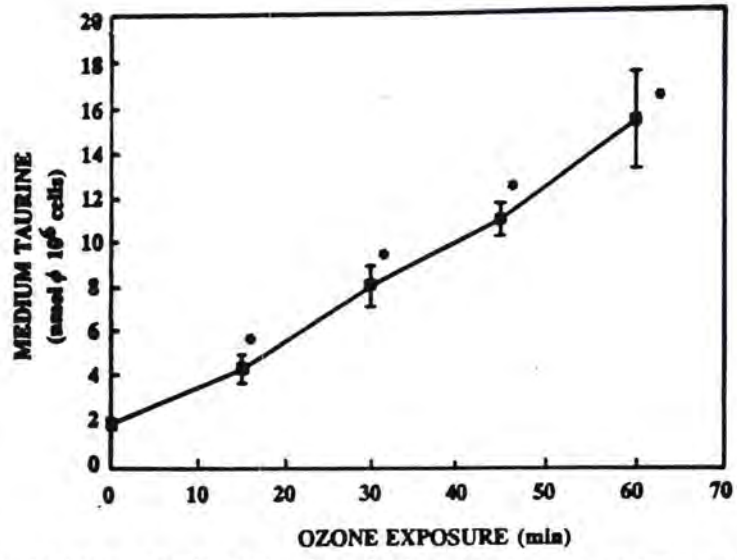

Figure 6. Taurine leakage into the medium from alveolar macrophages exposed to ozone for 0-60 min (mean ± SEM for N = 3 observations). *A significant increase from the value at 0 min at the $p < 0.05$ level (*t* test).

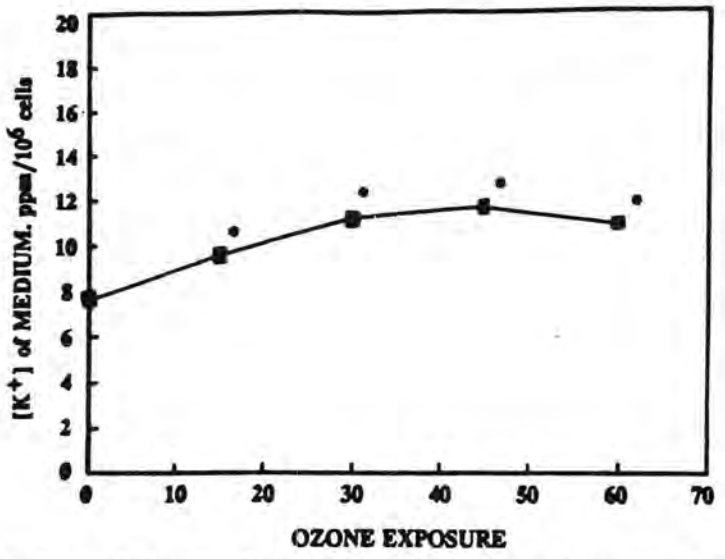

Figure 7. Potassium ion leakage into the medium from alveolar macrophages exposed to ozone for 0-60 min (mean ± SEM for N = 3 observations). *A significant increase from the value at 0 min at the $p < 0.05$ level (*t* test).

Protective Effects of Taurine on Ozone-Induced Lipid Peroxidation and Membrane Leakage in Isolated Rat Alveolar Macrophages

The actual intracellular taurine concentrations of alveolar macrophages preincubated in 0-500 μM taurine prior to ozone exposure are given in Figure 8. The taurine content of the cells increased with increasing extracellular taurine. This result was expected since rat alveolar macrophages have been shown to actively transport and accumulate this nutrient via a specialized sodium-taurine co-transport mechanism[11].

The free intracellular taurine concentrations of alveolar macrophages after exposure to ozone are given in Figure 9. Comparing data from Figures 8 and 9, cytoplasmic taurine rose by approximately 1.6, 1.3, 5, and 7-fold in ozone-exposed cells incubated at 0, 100, 250, and 500 μM extracellular taurine, respectively. This ozone-induced taurine mobilization was significant in macrophages supplemented with 250 and 500 μM extracellular taurine.

Ozone exposure of alveolar macrophages has been shown to decrease cellular viability, induce lipid peroxidation, decrease total ATPase and Na^+/K^+ ATPase levels, and cause leakage of glutathione and protein[12]. Data in Table 3 indicate that taurine enrichment decreased ozone-induced damage as measured by these cellular parameters. At 100 μM extracellular taurine (i.e., the approximated plasma level of this nutrient)[11], viability was increased by 38%, lipid peroxidation decreased by 70%, total ATPase increased by 113%, Na^+/K^+ ATPase increased by 62%, GSH leak decreased by 93%, and protein leak decreased by 67% compared to levels measured for ozone-exposed cells incubated in the absence of extracellular taurine. In the case of viability, lipid peroxidation, total ATPase, and GSH leak, this protection from oxidant injury was also significant at 250 μM and 500 μM; high extracellular taurine levels did not significantly elevate Na^+/K^+ ATPase or prevent protein leak.

As shown previously, ozone exposure of alveolar macrophages increased chemiluminescence, decreased cell recovery, and increased leakage of GSSG and potassium ions[12]. In the present study, taurine supplementation at any level failed to significantly alter these ozone-induced changes (data not shown).

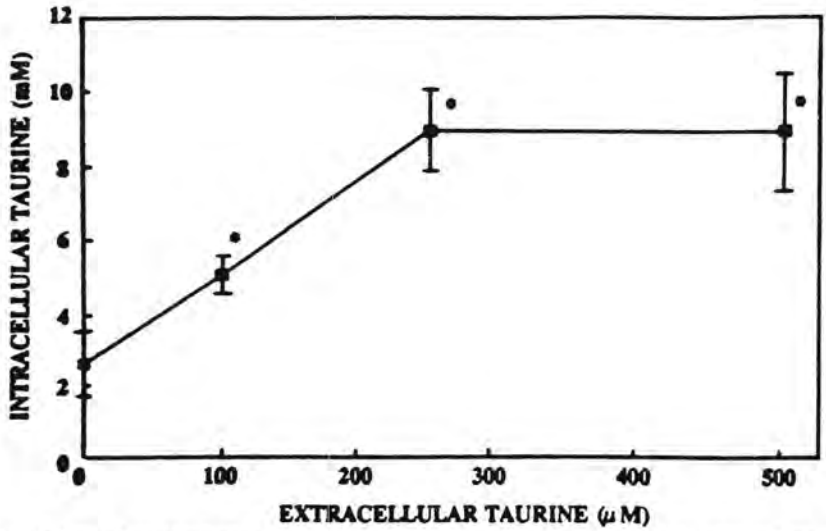

Figure 8. Effect of preincubation of rat alveolar macrophages with variable extracellular taurine concentrations on intracellular taurine content prior to ozone exposure. Accumulation of taurine is both time and concentration dependent. The asterisk (*) indicates a significant increase above the value for cells incubated in 0 μM taurine at the $p < 0.05$ level.

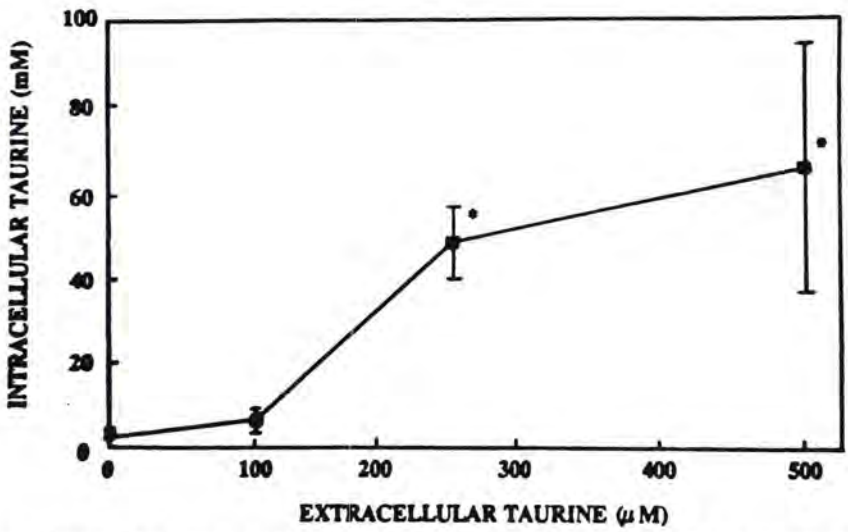

Figure 9. Intracellular taurine content of alveolar macrophages exposed to 0.45 $\pm$ 0.05 ppm ozone for 30 min. Cells were preincubated in media of different taurine concentrations at 37 °C for 30 min prior to being cultured at various taurine levels and exposed to ozone. At all extracellular taurine levels, free intracellular taurine rises in response to ozone exposure. The asterisk (*) indicates a significant increase from the value for unexposed cells supplemented at the respective extracellular taurine level ($p < 0.05$).

Table 3. Effect of taurine supplementation on ozone-induced injury to alveolar macrophages[a].

Parameter	Extracellular Taurine Concentration (μM)[b] 0	100	250	500
Cell Viability (%)	60.6 ± 3.0	84.1 ± 1.7*	86.0 ± 1.3*	78.3 ± 1.3*
Lipid Peroxidation (nmol MDA/10^6 cells)	4.6 ± 0.3	1.4 ± 0.4*	2.1 ± 0.9*	2.3 ± 0.7*
Total ATPase (nmol Pi/hr/10^6 cells)	30.8 ± 7.0	65.9 ± 4.0*	64.0 ± 2.0*	91.3 ± 4.2*
GSH Leak (μg/10^6 cells)	0.42 ± 0.14	0.03 ± 0.01*	0.04 ± 0.02*	0.05 ± 0.02*
Protein Leak (μg/10^6 cells)	15.9 ± 4.2	5.2 ± 1.4*	10.0 ± 1.0	11.4 ± 2.6
Na^+/K^+ ATPase (nmol/Pi/hr/10^6 cells)	4.4 ± 6.4	29.5 ± 3.4*	2.3 ± 3.0	4.3 ± 8.4

[a] Alveolar macrophages were preincubated in media of different taurine concentrations at 37°C for 30 min prior to being cultured at various taurine levels and exposed to ozone (0.45 ppm for 30 min at 25°C).

[b] Values are means ± standard errors from 3-8 separate experiments.

* Significantly different from the value at 0 μM taurine ($p < 0.05$).

DISCUSSION

Effects of In Vitro Ozone Exposure on Peroxidative Damage, Membrane Leakage, and Taurine Content of Rat Alveolar Macrophages

The results which we observed following exposure of isolated rat alveolar macrophages to ozone included decreased cell viability, increased resting and particle-stimulated chemiluminescence, inactivation of the Na^+/K^+ ATPase, appearance of lipid peroxides in the medium, increased leakage of protein, reduced and oxidized glutathione, taurine, and potassium ions into the medium, and increased intracellular taurine levels.

Our results are consistent with those of Van Der Zee et al.[16-18] who have described the toxic effects of ozone exposure on murine fibroblasts and human crythrocytes. When the fibroblasts were exposed to ozone (10 μmol/min for 0-80 min) in a system similar to the one we used, leakage of protein and potassium ions into the medium was shown to increase with length of exposure. The cellular level of reduced glutathione decreased with time, while that of oxidized glutathione initially increased (presumably due to the oxidation of GSH) and then decreased[16]. In erythrocytes, ozone exposure (4 μmol/min for 0-120 min conducted over a stirred cell suspension) resulted in lipid peroxidation, K^+ leakage, and a reduction in the cellular level of reduced glutathione[18].

Chronic exposure (3 hr/day, 5 days/week for 3 months) to nitrogen dioxide (0.5 ppm) or ozone (0.1 ppm) resulted in decreased viability of alveolar macrophages that were lavaged from the lungs of the exposed mice[19]. However, there was no effect of a short (2 hr) *in vitro* ozone (0.29-0.61 ppm) exposure on the viability of alveolar macrophages in monolayer culture in a chamber[20], or of a short (2-3 hr) *in vitro* NO_2 (1.3-17.0 ppm) or ozone (0.9-3.5 ppm) exposure on rat alveolar macrophage viability[21]. The latter *in vivo* exposure may have been too short to affect cell viability. In the former study, the petri dishes containing the cells were rotated such that the medium covered half of the cells for 30 sec and the remaining half of the cells were exposed to ozone for 30 sec. Thus, the exposures were more intermittent than in our study, where half of the cells were exposed approximately every 3 sec.

A decrease in cellular protein content and an increase in cellular GSH were observed in isolated alveolar macrophages from guinea pigs exposed to hyperoxia (85% for 90 hr)[22]. Exposure of isolated, perfused whole rat lung to hyperoxic conditions (0.4 x 10^6 Pa/min, 90 min) resulted in an increase in glutathione release from the organ[23]. Conversely, glutathione depletion of isolated rat alveolar macrophages has been shown to increase the susceptibility of the cells to oxidant injury[24]. Our results show a time-dependent leak of both GSH and GSSG upon exposure of alveolar macrophages to ozone. It should be noted that GSSG levels exceeded GSH levels. Much of the oxidized glutathione which was detected in the extracellular medium in our study may have been produced as a result of the reaction of ozone with reduced glutathione. This would explain the artifactually high GSSG/GSH ratios reported in this investigation.

Dowell et al.[25] were unable to detect lipid peroxidation in alveolar macrophages isolated from ozone-exposed rabbits (2-10 ppm for 3 hr or 0.5-2 ppm 8 hr/day for 8 days). However, lipid peroxidation has been strongly implicated to be the mechanism of toxic action of O_3 or NO_2 exposures in studies where isolated alveolar macrophages were preincubated with polyunsaturated fatty acids, arachidonic acid, vitamin C, or vitamin E, or in glutathione-depleted cells[26,27]. Pigmented alveolar macrophages have been observed in the lungs of animals exposed to NO_2[11] and presumably this pigmentation is similar to the accumulation of lipofuscin or ceroid pigment which results from lipid peroxidation in various organs of vitamin E-deficient animals.

Chemiluminescence is a function of the production of reactive oxygen metabolites in alveolar macrophages during the respiratory burst, the activation of which results in phagocytosis and bacterial killing[17]. Zymosan-stimulated chemiluminescence was increased by ozone to a greater extent than at rest, i.e., 13- and 8-fold, respectively, therefore, ozone treatment must activate the cells in some manner so that they are more reactive in the presence of particles. It is possible that this sensitization is mediated via ozone-induced changes in membrane structure, since substantial lipid peroxidation results from ozone exposure (Figure 2). The ability of particle exposure to enhance the production of reactive oxygen metabolites was maintained in exposed alveolar macrophages even after a 45-min treatment with ozone. These data suggest that exposure of alveolar macrophages to ozone may not have affected the bactericidal capacity of the surviving phagocytes. However, since the number of surviving macrophages had declined, the antibacterial defenses of the lung may be compromised by oxidant exposure. Indeed, the enhanced generation of reactive species by ozone-exposed alveolar macrophages (Table 1) would be expected to potentiate the oxidant damage in the lung.

In the present study, the cytoplasmic taurine level of alveolar macrophages increased in response to ozone exposure (Figure 9). Whether the alveolar macrophage has the ability to synthesize taurine has not, to our knowledge, been investigated. However, it is unlikely that induction of enzymes which synthesize taurine would occur as early as 15 min after exposure to ozone, when the increase in intracellular taurine was initially seen. Furthermore, no extracellular taurine was included in the medium in our experiments, so uptake of taurine cannot account for this result. Even so, the ozone-induced inactivation of the Na^+/K^+ ATPase which we observed would prevent uptake of taurine uptake since it is a sodium and energy-dependent process. Finally, two pools of intracellular taurine, a rapidly exchangeable free pool and a slowly exchangeable bound pool, have been identified[28]. Shifts of cytosolic to membrane-bound pools of taurine have been demonstrated to occur in response to magnesium deficiency[29,30]. Therefore, our results suggest the ozone-induced mobilization of taurine from bound stores. This mobilization and the resulting increase in cytoplasmic taurine may be significant steps in the process by which this nutrient acts as a possible antioxidant.

An increase in extracellular taurine levels was noted in this study (Figure 6). We have shown that the membrane of the alveolar macrophage is relatively leaky with

respect to taurine[11]. Therefore, initially some of the taurine detected in the extracellular medium may have leaked out of viable cells in response to an increase in cytoplasmic taurine. Later, the continued rise of taurine in the medium may have been a consequence of increasing membrane permeability and eventual lysis of macrophages as a result of prolonged ozone exposure.

Protective Effects of Taurine on Ozone-Induced Lipid Peroxidation and Membrane Leakage in Isolated Rat Alveolar Macrophages

The results of this study indicate that taurine may function as an antioxidant in rat alveolar macrophages at its physiological concentration of 100 μM (i.e., the plasma concentration of taurine in the rat). At this level of supplementation, taurine significantly protected alveolar macrophages from ozone-induced damage. That is, recovered cells demonstrated an increase in viability as judged by trypan blue exclusion, a decrease in lipid peroxidation, a decrease in the ozone-induced decline in total and Na^+-K^+ ATPase activity, and a lessening of the leak of reduced glutathione and protein. Protection against ozone-induced cell damage was less obvious at pharmacological concentrations of external taurine, since the decline in Na^+/K^+ ATPase and the leak of protein were not significantly prevented at 250 and 500 μM extracellular taurine. The suggestion that taurine may protect alveolar macrophages from oxidant injury agrees with its ability to protect bronchioles from oxidant injury due to NO_2 exposure[8].

Taurine has been proposed as both a direct and indirect antioxidant[1-3]. As a direct antioxidant, taurine would act to quench radicals derived from the interaction of ozone with membrane lipids. As an indirect antioxidant, taurine would act to stabilize the plasma membrane and thus prevent oxidant-induced increases in membrane permeability. In support of taurine's role as a membrane stabilizer, taurine has been shown to prevent Ca^{2+} influx in cat cerebral cortex resulting from treatment with ouabain[31] and to prevent K^+ leakage in dog heart[32]. In contrast, support for taurine as a direct antioxidant was given by Nakashima et al.[33] who reported that taurine was able to mitigate CCl_4-induced lipid peroxidation in rat liver. Data from the present study are consistent with both views, since taurine significantly reduced lipid peroxidation (direct effect) and significantly increased membrane integrity (indirect effect).

The data on intracellular taurine concentrations after exposure to ozone deserve attention. In our previous study of the time course of metabolic changes occurring in alveolar macrophages during ozone exposure, free cytoplasmic taurine increased with ozone exposure. Therefore, we concluded that taurine was mobilized from cellular bound stores to the free state in response to oxidant injury[12]. In the present study, extremely high intracellular taurine concentrations resulted in alveolar macrophages incubated with 250 μM or 500 μM taurine prior to ozone exposure. There are two possible explanations for this effect: either (1) cells incubated in high taurine levels were stimulated to increase their rate of taurine uptake from the medium in response to ozone exposure; or (2) the cells were mobilizing taurine from bound stores to the free state in response to ozone exposure. Taurine uptake is Na^+ and energy dependent[11]. Since Na^+-K^+ ATPase activity in ozone-exposed cells at 250 or 500 μM taurine was very low, the size of the inwardly directed concentration gradient for Na^+ should have decreased. Thus, it is unlikely that taurine uptake had increased. Therefore, the second explanation seems more likely.

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