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Research Article

Airway secretions of cystic fibrosis patients were found to contain high concentrations of taurine, which decreased with antibiotic therapy during acute respiratory exacerbations. Taurine, in a 1:1 molar ratio with HOCl/OCl-, caused a 10-fold increase in the amount of HOCl/OCl- needed to induce cytotoxicity to the cat lung epithelial cell line, AKD. Although DMSO protected cells against HOCl/OCl(-)-mediated injury, the presence of an equimolar concentration of taurine with HOCl/OCl- prevented DMSO from protecting cells and sulfhydryl groups against oxidation, suggesting the formation of taurine chloramines. Spectral properties confirmed the formation of monochloramines and dichloramines. Chloride-free buffer, DIDS, and low temperature (4 degrees C) each protected the cells against taurine/HOCl/OCl-, indicating that taurine chloramine uptake through anion transport pathways was required to induce cytotoxicity. A molar excess of taurine inhibited cytotoxicity, to induce cytotoxicity. A molar excess of taurine inhibited cytotoxicity, by decreasing taurine dichloramines and increasing the formation of less toxic taurine monochloramines. We conclude that taurine can protect lung epithelial cells by converting HOCl/OCl- to anionic monochloramines, but that taurine dichloramines can be toxic to respiratory epithelial cells through mechanisms that depend upon epithelial cell anion transport.

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Taurine Modulation of Hypochlorous Acid–induced Lung Epithelial Cell Injury In Vitro

Role of Anion Transport

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Abstract

Airway secretions of cystic fibrosis patients were found to contain high concentrations of taurine, which decreased with antibiotic therapy during acute respiratory exacerbations. Taurine, in a 1:1 molar ratio with $HOCl/OCl^-$, caused a 10-fold increase in the amount of $HOCl/OCl^-$ needed to induce cytotoxicity to the cat lung epithelial cell line, AKD. Although DMSO protected cells against $HOCl/OCl^-$–mediated injury, the presence of an equimolar concentration of taurine with $HOCl/OCl^-$ prevented DMSO from protecting cells and sulfhydryl groups against oxidation, suggesting the formation of taurine chloramines. Spectral properties confirmed the formation of monochloramines and dichloramines. Chloride-free buffer, DIDS, and low temperature (4°C) each protected the cells against taurine/$HOCl/OCl^-$, indicating that taurine chloramine uptake through anion transport pathways was required to induce cytotoxicity. A molar excess of taurine inhibited cytotoxicity, by decreasing taurine dichloramines and increasing the formation of less toxic taurine monochloramines. We conclude that taurine can protect lung epithelial cells by converting $HOCl/OCl^-$ to anionic monochloramines, but that taurine dichloramines can be toxic to respiratory epithelial cells through mechanisms that depend upon epithelial cell anion transport. (*J. Clin. Invest.* 1994. 93:606–614.) Key words: cystic fibrosis • bronchiectasis • oxidants • myeloperoxidase • chloramines

Introduction

Taurine (2-aminoethanesulfonic acid) is a unique β-amino acid that contains a sulfate group rather than a carboxylic acid group and thus is not incorporated into proteins (1). Although taurine does not participate in protein synthesis, it is the most abundant free amino acid in the cytoplasm of most cells. Taurine has several critical functions in cell metabolism, including osmoregulation, membrane protection, antioxidant defense, and regulation of cellular calcium homeostasis (1, 2). It also plays a major role in conjugating bile acids, and thus reducing bile acid lithogenicity. Patients with cystic fibrosis (CF)[1] are relatively deficient in taurine, a condition reflected by a high bile acid glycine/taurine ratio (3). The cause of this deficiency is thought to be the excessive loss of taurine from the digestive tract (4).

Human neutrophils and lung epithelial cells have particularly high concentrations of taurine at 19 and 14 mM, respectively (5, 6). Although the concentration of taurine in extracellular fluids is normally low, cystic fibrosis airway secretions are rich in activated neutrophils, neutrophil-derived products, and cell debris, a situation that could conceivably favor high taurine concentrations at the lung epithelial surface.

Patients with CF also have very high myeloperoxidase concentrations in their sputum (7). Myeloperoxidase catalyzes the reaction of hydrogen peroxide with chloride to form hypochlorous acid/hypochlorite ($HOCl/OCl^-$). Hypochlorous acid/hypochlorite can react with other molecules to form a variety of chlorinated oxidants (8). At least half of the neutrophil-derived chloramines result from the reaction of $HOCl/OCl^-$ with endogenous taurine (9). Although taurine is generally thought of as an antioxidant, previous studies have demonstrated that even in the presence of high concentrations of taurine, the transformation of $HOCl/OCl^-$ to taurine chloramines does not reduce the oxidative potential of $HOCl/OCl^-$ against various chemical groups such as thiols and thioethers (10).

Because of their hydrophilic nature, it has been suggested that taurine chloramines are restricted to the extracellular milieu, thus limiting their cytotoxic potential (8). However, taurine is a zwitterion, which, when oxidized to taurine chloramine, becomes an organic anion. Thomas et al. have shown that taurine chloramines can be taken up by red blood cells through the anion exchanger and rapidly reduced to taurine through the glutathione system (11). Since the red blood cell membrane is impermeable to taurine, reduced taurine is trapped within the cytoplasm and does not accumulate in the extracellular space. When taurine chloramine uptake exceeds red blood cell glutathione-dependent antioxidant defenses, cytotoxicity ensues.

Anion exchange has also been observed in lung epithelial cells (12). However, the lung epithelial cell differs from the red blood cell in that it has a lower glutathione content, and is permeable to taurine through a sodium-dependent transport system (6, 13, 14). The potential effects of taurine chloramine

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1. *Abbreviations used in this paper:* BAL, bronchoalveolar lavage; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; CI, cytotoxicity index; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid; DTNB, dithiobis-nitrobenzoic acid; EBSS, Earle's balanced salt solution; $HOCl/OCl^-$, hypochlorous acid/hypochlorite; HPBR, Hepes phosphate-buffered Ringer's solution; Σ_M, molar extinction coefficient.

uptake by lung epithelial cells through ion transport systems is unknown. This study was designed to (*a*) determine whether high taurine concentrations are present at the epithelial surface of the lung in cystic fibrosis, (*b*) determine whether taurine can modulate $HOCl/OCl^-$-mediated lung epithelial cell cytotoxicity, and (*c*) evaluate the role of ion transport in the modulation of lung epithelial cell cytotoxicity by taurine.

Methods

Patient population. 22 subjects with cystic fibrosis (9 female, 13 male, age 20±2 yr) were studied while off antibiotic therapy, and 8 were also studied during 16 episodes of hospitalization with IV antibiotic treatment (ceftazidime and tobramycin) for acute respiratory exacerbations. Respiratory exacerbation requiring hospitalization was defined as a deterioration in two or more of the following parameters: increased cough and sputum production, weight loss, increased dyspnea and/or fever. 10 normal non-smokers (age 22±1 yr), 9 individuals with smoking-related chronic bronchitis (9 male, age 66±5 yr), and 7 with non-CF bronchiectasis (2 female, 5 male, age 44±10 yr) were also studied. Sputum was collected from all subjects with disease. Since healthy individuals did not produce sputum, epithelial lining fluid was obtained by bronchoalveolar lavage (BAL) as previously described (15). Based on the urea dilution method described by Rennard et al. (16), 1 ml epithelial lining fluid (ELF) was recovered per 111.9±16.2 ml bronchoalveolar lavage fluid. One CF patient underwent BAL, but this procedure was complicated by fever and hemoptysis within 24 h, thus leading to the decision not to perform BAL in subsequent CF patients.

Taurine assay. 10 ml of BAL were lyophilized and reconstituted in 1 ml of 0.1 M HCl. Sputum was weighed and acidified with 1 vol (vol/wt) of 0.1 M HCl. The acidified BAL and sputum were then heated at 100°C for 1 min to precipitate protein, and centrifuged at 15,000 *g*, 15 min. Taurine was assayed in the supernatant by reversed-phase HPLC. Briefly, 1 ml sample supernatant was passed over 0.8 ml of the anion exchange resin (AG 1-X8; Bio-Rad Laboratories Ltd., Mississauga, Ont., Canada) and 0.8 ml of the cation exchange resin (AG 50W-X8; Bio-Rad Laboratories Ltd.), and eluted in 2 ml H_2O. Since taurine is a zwitterion, it was not retained on the resins, and recovered in the first 2 ml of elution. Quantitation of taurine in the eluate was performed by HPLC as described by Porter et al. (17). The presence of taurine in the elution volume of the cation/anion exchange resins was also confirmed by TLC on a 150-Å pore-sized silica gel (No. LK5D; Whatman Inc., Clifton, NJ) in a solvent comprised of butanol/acetic acid/water in a ratio of 13:3:5. A single ninhydrin-reactive band migrating the same distance as the taurine standard, was observed in the CF sputum, thus confirming that the peak observed on HPLC analysis was taurine (data not shown). Results for normal subjects are expressed as μmol/g of 10-fold concentrated BAL. Results in sputum are expressed as μmol/g sputum. Taurine was also assayed in the plasma of 9 normal subjects. The lower limit of detectability was 25 nmol/g. To distinguish between intracellular and extracellular taurine, sputum from 12 CF patients was liquefied with 1 mg/g DNAase at 37°C for 30 min, centrifuged at 500 *g*, 20 min, and taurine measured in the acidified supernatant. To determine whether saliva contributed to the taurine detected in sputum, taurine was measured in the saliva of four normal and three CF subjects. Taurine concentrations were also determined in *Pseudomonas aeruginosa* strains derived from three different patients with CF. The strains were originally cultured on PA agar (Difco Laboratories, Detroit, MI). Isolated colonies were subcultured in LB broth (Difco Laboratories) for 48 h, and bacteria obtained by centrifugation. Taurine was measured in the lysates of 10^8 bacteria suspended in 1 ml 0.1 M HCl. We also attempted to detect taurine chloramines in CF sputum. Sputum from CF patients was immediately treated with 0.1 mg/ml DNAase (Sigma Chemical Co., St. Louis, MO) for 30 min at 37°, centrifuged at 25,000 *g*, 15 min, and the supernatant was ultrafiltered through a membrane (No. PM-10; Amicon Co., Lexington, MA). The ultrafiltrate was analyzed for the presence of chloramines using 5-thio-2-nitrobenzoic acid (18).

Cytotoxicity assays. Modulation of lung epithelial cell oxidant injury by taurine was assessed using the cat lung epithelial cell line, AKD (CCL 151; American Type Culture Collection, Rockville, MD; reference 19). The cat lung epithelial cell line rather than primary cell cultures were used, since many of the experiments required large numbers of cells. To help assess the effect of taurine on $HOCl/OCl^-$ cytotoxicity to human upper airway epithelial cells, experiments were also performed on human epithelial cell lines derived from CF and non-CF nasal polyps, as described below. AKD cells were grown to confluence in 24-well culture plates (Linbro Chemical Co., New Haven, CT) in 0.5 ml DME (Gibco Diagnostic Laboratories, Grand Island, NY) supplemented with 10% calf serum, in 10% CO_2 at 37°C. The cells were labeled with sodium chromate (^{51}Cr; Amersham Canada Ltd., Oakville, Ont.; 410 μCi/μg, 10 μCi/ml) overnight and subsequently washed three times with PBS. Earle's balanced salt solution (EBSS) 0.5 ml, was added to each well and the cells were incubated in the presence of either EBSS alone or various test conditions (see below) for 7 h, in 5% CO_2 at 37°C. The amount of ^{51}Cr released in the supernatant of each test condition was then quantitated. Results are expressed as a cytotoxicity index (CI) determined with the formula: $CI = (A - B)/(C - B) \times 100$, where A = dpm of the test sample, B = dpm of spontaneous release in EBSS alone, and C = dpm of 1% Triton-X treated cells as previously described (20).

Preparation of $HOCl/OCl^-$. For all cytotoxicity experiments, NaOCl (BDH Chemicals, Toronto, Ont., Canada) was diluted immediately before use, in EBSS to the desired concentration. The concentration of $HOCl/OCl^-$ was determined at the start and at the end of the incubation period by quantitating the oxidation of the yellow sulfhydryl dye, 5-thio-2-nitrobenzoic acid to its colorless disulfide, 5-5′-dithiobis (2-nitrobenzoic acid) in a spectrophotometer (model DU-7, Beckman Instruments Canada Inc., Mississauga, Ont., Canada) as described by Thomas (21). A molar extinction coefficient (Σ_M) of 13,600 was assumed for 5-thio-2-nitrobenzoic acid (22). $HOCl/OCl^-$ concentrations were found to be stable throughout the incubation period.

Reactivity of oxidants with dimethylsulfoxide. Since DMSO is an excellent hypochlorous acid scavenger, but does not react with chloramines (18), we used DMSO to help determine whether cytotoxicity and thiol oxidation in the presence of taurine and $HOCl/OCl^-$, was induced by residual $HOCl/OCl^-$ or by newly formed chloramines. $HOCl/OCl^-$, 0–1.75 mM, and an equimolar concentration of taurine were incubated alone or in the presence of 1 mM DMSO, with either the lung epithelial cells or a solution of 80 μM reduced dithiobisnitrobenzoic acid (DTNB). Cytotoxicity was determined after a 7-h incubation at 37°C, as described above. Thiol oxidation was measured after a 15-min incubation at 25°C, using the DU-7 spectrophotometer (Beckman Instruments Canada Inc.) at 412 nm (18).

Effect of DIDS on taurine/$HOCl/OCl^-$-mediated cytotoxicity. To determine the role of the anion transport system(s) in mediating lung epithelial cell cytotoxicity, 100 μM of freshly prepared 4,4′diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), was added to the labeled cells in the presence of 0–1.75 mM taurine/$HOCl/OCl^-$ (1:1 molar ratio). The CI was determined after a 7-h incubation at 37°C. To determine whether the protective effect of DIDS against taurine chloramines was reversible, cells were incubated with 100 μM DIDS for 30 min, washed three times in EBSS, and further incubated with 0–2 mM taurine/$HOCl/OCl^-$ (1:1 molar ratio) for 7 h at 37°C, 5% CO_2, followed by quantitation of the CI. The ability of DIDS to scavenge either $HOCl/OCl^-$ or taurine chloramines was evaluated by adding 100 μM DIDS to 100 μM $HOCl/OCl^-$ and taurine in a 1:1 molar ratio in EBSS at 37°C, 5% CO_2. After 5-min and 6-h incubation periods, 50 μl of each solution was added to a final volume of 600 μl of 80 μM 5-thio-2-nitrobenzoic acid. Oxidation was quantitated in the DU-7 spectrophotometer (Beckman Instruments Canada Inc.) at 412 nm (18).

Effect of temperature on oxidant injury. The effect of temperature on lung epithelial cell susceptibility to both hypochlorous acid and

taurine/hypochlorous acid-mediated injury was assessed to evaluate the role of temperature-sensitive anion transport systems. The ^{51}Cr-labeled cells were incubated in the presence of either 0–400 μM HOCl/OCl^- or 0–2 mM taurine/(HOCl/OCl^-), with a 1:1 molar ratio, at 37 and at 4°C. At the end of the incubation period, supernatants were collected and ^{51}Cr release was quantitated to determine the CI as described above.

Evaluation of taurochloramine uptake by lung epithelial cells. To evaluate the role of anion transport in the uptake of taurochloramine, cells were incubated with 1 mM [^{3}H]taurine (28 Ci/mmol, 10 μCi/ml; Amersham Canada Ltd.) and 1 mM HOCl/OCl^- for 2 h in the following conditions: (*a*) Hepes phosphate-buffered Ringer's solution (HPBR) comprised of 5 mM Hepes, 140 mM NaCl, 3.3 mM K_2HPO_4, 0.83 mM KH_2PO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM D-glucose, pH 7.4, at 37°; (*b*) HPBR and 100 μM DIDS at 37°C; (*c*) chloride-free HPBR in which NaCl, $CaCl_2$, and $MgCl_2$ were replaced by 140 mM sodium gluconate, 1 mM $CaSO_4$, and 1 mM $MgSO_4$ at 37°C; (*d*) chloride-free HPBR and 100 μM DIDS at 37°C; and (*e*) HPBR at 4°C. At the end of the incubation period, the cells were washed three times, and lysed in 1% Triton-X. The radioactivity within the lysates was quantitated to determine the uptake of taurochloramines.

Effect of an excess molar ratio of taurine on cytotoxicity and thiol oxidation. Taurine, in the amount of 2–6 mM, was added to 2 mM HOCl/OCl^-, and subsequently incubated with the ^{51}Cr-labeled cells to determine the effect of a molar excess of taurine on HOCl/OCl^-–dependent lung epithelial cell cytotoxicity. Thiol oxidation in the presence of excess taurine was determined by reacting 0–4 mM taurine and 2 mM HOCl/OCl^- with 80 μM DTNB for 15 min, at 25°C and subsequently measuring the absorbance of each solution at 412 nm (18).

Spectrophotometric characterization of the taurine/hypochlorous acid products. The absorbance of solutions of EBSS containing 2 mM HOCl/OCl^- alone or in the presence of 2–8 mM taurine was quantitated between the wavelengths 220–340 nm in a scanning spectrophotometer (Beckman Instruments Canada Inc.).

Extracellular bicarbonate and taurochloramine cytotoxicity. Since toxic taurine dichloramine formation is favored at acid pH (18), we evaluated the effect of extracellular sodium bicarbonate on the cytotoxicity of taurochloramines. Cells were incubated with 2–6 mM taurine, and 2 mM HOCl/OCl^- in EBSS containing either 26 or 2.6 mM sodium bicarbonate at 37°C in 10% CO_2 for 7 h. The pH of each solution was determined at the end of the incubation period and the CI was measured as described above.

Cell glutathione content. To determine whether alterations in glutathione levels within cells exposed to different pH's and Cl^- concentrations may have played a role in the susceptibility to oxidant injury, glutathione was determined in the cells. Confluent AKD cells were incubated in either EBSS, EBSS with a bicarbonate concentration of 2.6 mM, HPBR, or chloride-free HPBR for 4 h at 37°C. At the end of the incubation period, the cells were washed three times, lysed in 1% Triton-X, centrifuged, and the supernatants were collected to quantitate total protein and glutathione as previously described (23).

Effect of wild-type and mutant CFTR expression on taurochloramine cytotoxicity. Transformed human respiratory epithelial cell lines were obtained as a gift from Dr. Manuel Buchwald. Briefly, an SV-40 transformed cell line (NP-34), not expressing CFTR cDNA and derived from human nasal polyps, was used to transfer either wild-type or mutant ΔF508 CFTR cDNA. Transfection of CFTR cDNA was performed using a retroviral vector containing CFTR cDNA and a neomycin resistance gene. Clones expressing wild-type (NP-13.21) and mutant ΔF508 CFTR (NP-56.8, NP-56.12, and NP-56.13) were selected in neomycin-containing media. Chloride permeability of each cell line was studied by incubating 10^6 cells/well in chloride-free buffer (119 mM Na-gluconate, 1.2 mM K_2HPO_4, 0.6 mM KH_2PO_4, and 25 mM $NaHCO_3$) for 1 h at 37°C, 5% CO_2, and subsequently adding 1 μCi ^{36}Cl (3.43 MBq/ml; Amersham Canada Ltd.). The cells were then stimulated with 10^{-6} M forskolin and the amount of chloride secreted in supernatants was quantitated by gamma counting at 0–5 min. Cytotoxicity assays using taurine and HOCl/OCl^- (1:1 ratio, 0–8 mM) in the presence and absence of 100 μm DIDS were performed as described above.

Statistical analysis. The data are expressed as the mean±SEM. In vitro studies were performed in triplicate and repeated at least three times. A single-factor repeated-measures ANOVA was used to analyze data involving more than two groups (24). When F values were significant at $P < 0.05$, comparisons between groups were made using the least significant difference (LSD) test. Linear regression analysis of taurine levels as a function of days of antibiotic therapy was based on a linearity-log model. A P value < 0.05 was considered significant.

Results

Taurine in airway secretions. Taurine was undetectable in respiratory secretions obtained by BAL from 10 normal individuals (< 25 nmol/g of 10-fold concentrated BAL fluid, or expressed as ELF concentration, < 250 μM). In contrast, taurine was readily detected in the unconcentrated BAL of one CF patient at 544.8 nmol/g BAL fluid. Sputum samples from each of the 22 patients with CF were found to have high taurine concentrations, but this finding was not specific to CF, as individuals with bronchiectasis and chronic bronchitis also had increased levels (in the case of CF patients, sputum taurine measured 3.68±0.50 μmol/g; for non-CF bronchiectasis patients the level was 1.99±0.71 μmol/g; for chronic bronchitis patients the level was 0.92±0.29 μmol/g; and for chronic bronchitis vs. CF we note that $P < 0.005$, as graphed in Fig. 1). The concentration of taurine in the acellular supernatant of CF sputum was 94% of the total suptum concentration. Saliva taurine concentrations were 89±15 μM, a value similar to that of normal plasma at 99.3±9.9 μM, but 40-fold less than the mean CF sputum taurine concentration. Taurine was undetectable in *P. aeruginosa*. A strong inverse correlation was found between the CF sputum taurine concentrations and the number of days of intravenous antibiotics (regression equation: sputum taurine (μmol/g) = $-3.5 \times \log$(days of antibiotic therapy) + 5.8; $r^2 = 0.89$, $P < 0.0001$; Fig. 2). Taurine chloramines as measured by the oxidation of 5-thio-2-nitrobenzoic acid, were undetectable in CF sputum.

Effect of taurine on HOCl/OCl^- cytotoxicity. HOCl/OCl^- induced lung epithelial cell cytotoxicity in a dose-dependent fashion, with a maximal effect at ~ 200 μM. Consistent with the knowledge that DMSO scavenges HOCl/OCl^-, cytotoxic-

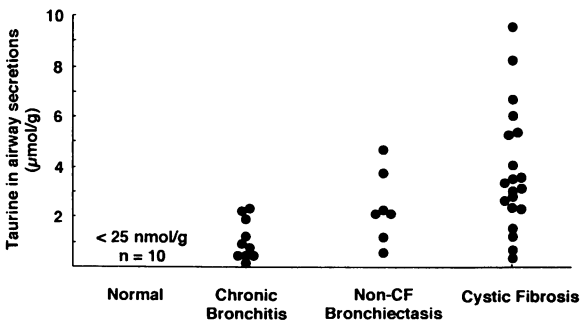

Figure 1. Taurine content of respiratory secretions from normal individuals and patients with chronic bronchitis, bronchiectasis, and cystic fibrosis. Taurine in respiratory secretions obtained by BAL from normal subjects was undetectable. Measurements in the disease groups were performed on sputum and are expressed relative to weight.

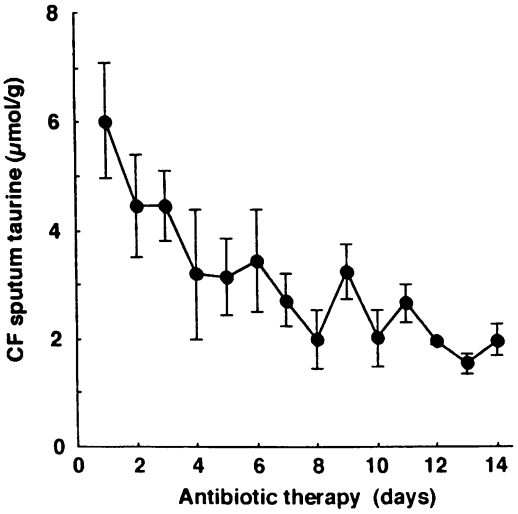

Figure 2. Effect of intravenous antibiotic therapy on the sputum taurine content in patients with acute exacerbation of their CF lung disease. Data represent the mean±SEM of 16 determinations made in 8 CF individuals.

ity was completely inhibited at all concentrations of HOCl/OCl^- by 1 mM DMSO (400 μM HOCl/OCl^-, CI = 60±8%; with 2 mM DMSO, CI = 4±1%; $P < 0.001$; Fig. 3 *A*). In the presence of an equimolar amount of taurine, the concentration of HOCl/OCl^- needed to induce maximal cytotoxicity was approximately 10-fold higher, at 1.75 mM (Fig. 3 *B*). Dimethyl sulfoxide had no protective effect against any concentration of HOCl/OCl^- in the presence of taurine, thus suggesting that taurine had transformed HOCl/OCl^- to taurine chloramines, molecules that are not scavenged by DMSO (18). Consistent with this concept, DMSO protected sulfhydryl groups against HOCl/OCl^- oxidation, but had no effect on thiol oxidation by HOCl/OCl^- in the presence of taurine (Fig. 4). Since taurine chloramines are small anionic molecules that can enter cells through anion exchange, we verified the ability of the anion exchange inhibitor, DIDS, to inhibit cytotoxicity in the presence of HOCl/OCl^- alone, and in the presence of an equimolar amount of taurine. DIDS at 100 μM was ineffective against HOCl/OCl^- but provided complete protection to cells against HOCl/OCl^-/taurine–mediated cytotoxicity (Fig. 3). The protective effect of DIDS was not due to scavenging of taurine chloramines, since 100 μM DIDS did not protect the sulfhydryl dye, 5-thio-2-nitrobenzoic acid, against oxidation as quantitated by the formation of 5-5′-dithiobis (2-nitrobenzoic acid; DTNB) in the presence of HOCl/OCl^-/taurine (in the case with no oxidant, DTNB = 2.6±10.2 μM; in the case with 100 μM taurine chloramine, DTNB = 194.0±3.6 μM; in the case with 100 μM taurine chloramine + 100 μM DIDS, DTNB = 204.8±2.4 μM; $P > 0.2$ for taurine chloramine with and without DIDS). The protective effect of DIDS persisted after the DIDS had been thoroughly washed from the cells (in the case of 2 mM taurine chloramine, CI = 55.7±2.0%; in the case of 100 μM DIDS, cells washed + 2 mM taurine chloramine, CI = 10.6±2.3%; $P < 0.001$).

Effect of temperature on HOCl/OCl^-/taurine cytotoxicity. Since the uptake of anions through the anion exchanger is markedly reduced at 4°C, we verified the effect of temperature on cytotoxicity. At 4°C, there was no decrease in HOCl/OCl^-–mediated cytotoxicity, but rather a slight increase compared to that observed at 37°C (Fig. 5 *A*). In contrast, cytotoxicity from HOCl/OCl^-/taurine was completely inhibited at 4°C (Fig. 5 *B*).

Modulation of cytotoxicity by chloride. In the presence of a chloride-containing Hepes-phosphate buffer, HOCl/OCl^-/taurine, in a 1:1 molar ratio, clearly induced epithelial cell damage (Fig. 6). Removal of chloride from the HPBR buffer did not affect the spontaneous release of ^{51}Cr from the cells, but did reduce the cytotoxicity induced by HOCl/OCl^-/taurine. The degree of protection afforded by removal of chloride was slightly less than that of 100 μM DIDS. The protective effect of chloride-free medium was not due to an increase in cell GSH, since chloride-free HPBR caused a slight decrease in cell GSH (control, GSH = 6.27±0.09 ng/mg protein; chloride-free HPBR, GSH = 5.07±0.06 ng/mg protein, $n = 6$, $P < 0.01$). When 100 μM DIDS was added to the cells in chloride-free buffer, there was an additive effect of protection against HOCl/OCl^-/taurine.

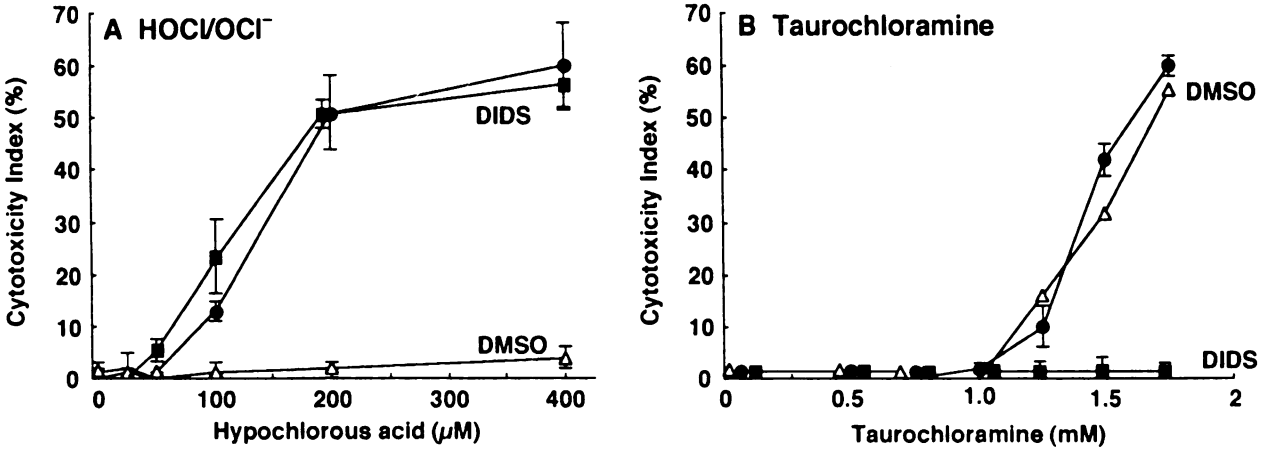

Figure 3. Comparison of the cytotoxicity induced to the lung epithelial cell line, AKD, by either (*A*) HOCl/OCl^- alone or (*B*) in the presence of an equimolar concentration of taurine (taurochloramine). The effect of 1 mM DMSO (△) on HOCl/OCl^- and taurochloramine-dependent cytotoxicity was also examined, as was that of 100 μM DIDS (■) in the presence of either HOCl/OCl^- or taurochloramines. Each data point represents the mean±SEM of triplicate assays performed at least three times.

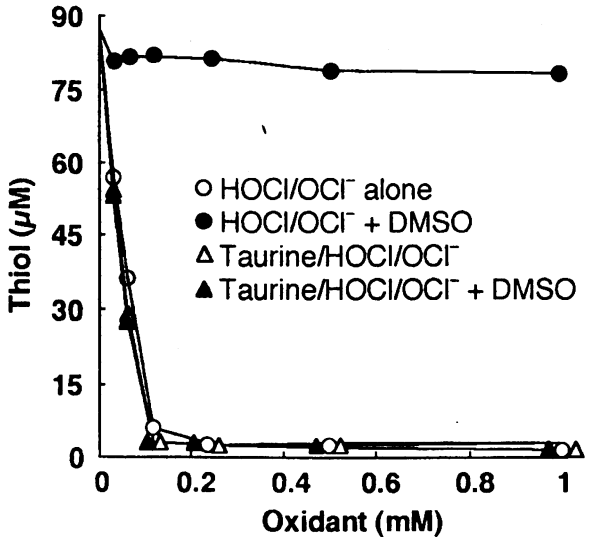

Figure 4. Thiol oxidation induced by $HOCl/OCl^-$ alone or in the presence of taurine. $HOCl/OCl^-$ (0–1 mM) alone, and in the presence of an equimolar concentration of taurine, were incubated in the presence and absence of 1 mM DMSO at 25°C for 15 min. Subsequently, a solution of 80 μM reduced DTNB was added and thiol oxidation was measured after a 15-min incubation at 25°C, using a spectrophotometer at 412 nm, and assuming Σ_M = 13,600. Results represent the mean of five experiments.

Uptake of taurine chloramines by lung epithelial cells. The lung epithelial cells were clearly capable of taking up taurine chloramines (Table I). The uptake of taurine chloramines was reduced by 21 and 28% in the presence of chloride-free buffer and 100 μM DIDS, respectively ($P < 0.05$ each compared to control buffer). When 100 μM DIDS was added to chloride-free buffer, taurine chloramine uptake was inhibited by 66%, a degree of inhibition similar to that induced by incubating the cells in control buffer at 4°C (61% inhibition).

Taurine in excess of a 1:1 molar ratio with HOCl. The addition of taurine in excess of a 1:1 molar ratio with HOCl inhibited cytotoxicity in a dose-dependent fashion (Fig. 7). To evaluate the mechanism by which a molar excess of taurine protects lung epithelial cells against HOCl-mediated toxicity, we examined the ability of taurine to prevent thiol oxidation by $HOCl/OCl^-$. Taurine, up to a 4:1 molar excess, did not inhibit the ability of $HOCl/OCl^-$ to oxidize thionitrobenzoic acid, and actually enhanced the oxidative capacity of $HOCl/OCl^-$ likely by converting the relatively unstable $HOCl/OCl^-$ to the more stable chloramines (50 μM $HOCl/OCl^-$, reduced DTNB = 35±3% of control; 200 μM taurine/50 μM $HOCl/OCl^-$, reduced DTNB = 4±1% of control, $P < 0.01$, Fig. 8). Since taurine did not decrease the potential of $HOCl/OCl^-$ to oxidize sulfhydryl groups, we evaluated the capacity of taurine to transform $HOCl/OCl^-$ into the less toxic monochloramine species, by examining the spectral properties of $HOCl/OCl^-$ in the presence of taurine. A solution of 2 mM $HOCl/OCl^-$ was found to absorb light maximally at a wavelength of 291 nm. The addition of 2 mM taurine to 2 mM $HOCl/OCl^-$ markedly reduced absorption at 291 nm and increased absorbance at both 252 and at 205 nm. Increasing taurine to 4 mM reduced the absorbance at 205 nm and increased absorbance at 252 nm (Fig. 9).

Bicarbonate depletion and taurine/HOCl/OCl⁻-mediated cytotoxicity. To further investigate the concept that taurine protected the lung epithelial cells through the conversion of dichloramines to monochloramines, acidification of the extracellular milieu to a pH of 6.4 was induced by reducing the bicarbonate concentration to 10% of control medium. Cellular glutathione content was decreased 12% by incubation in low bicarbonate medium (control medium: GSH = 10.15 nmol/mg protein; low bicarbonate medium: GSH = 8.96±0.16 nmol/mg protein, $P < 0.05$) but spontaneous release of ^{51}Cr was unchanged by reduction of the bicarbonate concentration. Acidification of the extracellular medium markedly increased the amount of taurine needed to reduce the cytotoxicity index by 50% from 0.4 to 3.2 mM ($P < 0.01$, Fig. 10).

Effect of CFTR expression in human respiratory epithelial cells on cytotoxicity. Chloride permeability of cells transfected with wild-type CFTR cDNA was increased compared to cells transfected with ΔF508 CFTR (Fig. 11 *A*, NP13.21 vs. NP56.8, NP56.12, and NP56.13, $P < 0.01$). The transformed nasal polyp cell line, NP-34, which did not express CFTR, was found to be slightly more resistant to taurochloramine-induced cyto-

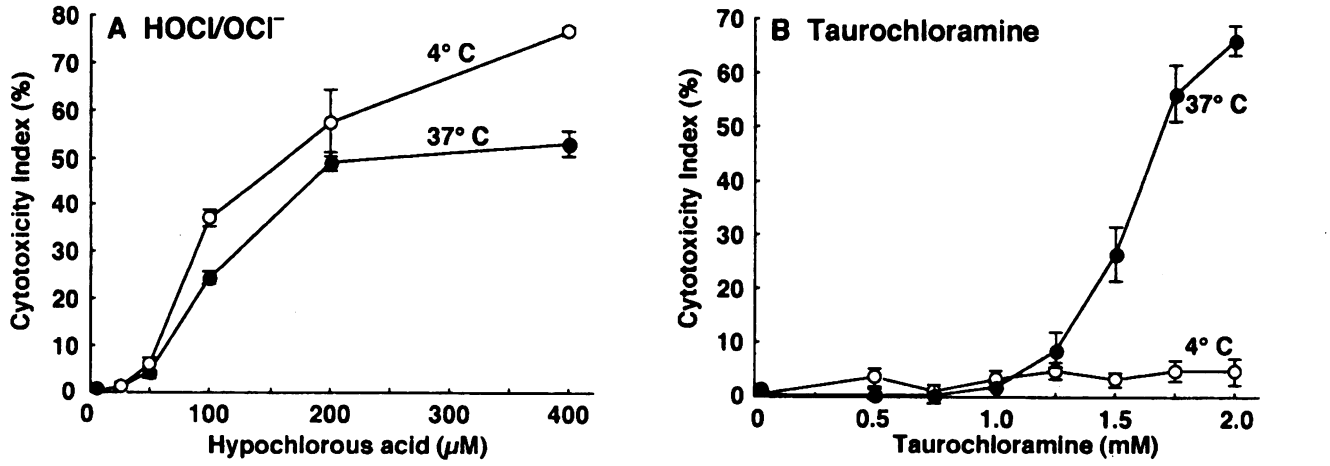

Figure 5. Effect of temperature on the cytotoxicity induced to the lung epithelial cell line, AKD, by either (*A*) $HOCl/OCl^-$ alone or (*B*) in the presence of an equimolar concentration of taurine. Cytotoxicity experiments were performed as described in the text, and incubated for 7 h at either 37°C (●) or 4°C (○). Results represent the mean±SEM of triplicate assays performed four times.

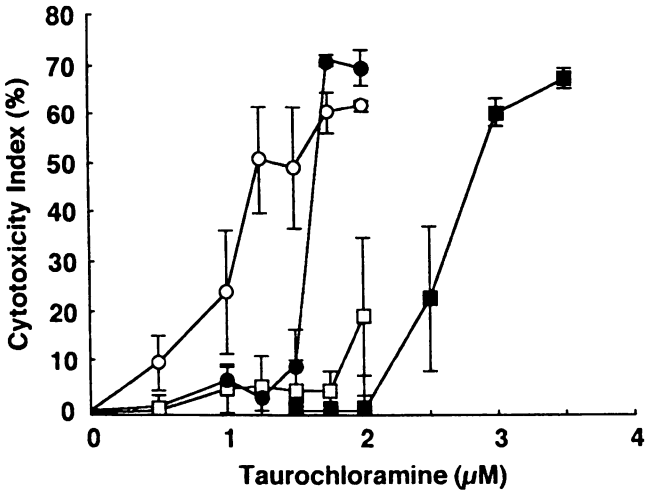

Figure 6. Dose–response of cytotoxicity to AKD cells induced by equimolar concentrations of $HOCl/OCl^-$ and taurine alone (○), in the presence of a chloride-free buffer (●), 100 μM DIDS (□), and 100 μM DIDS in chloride-free buffer (■). Results represent the mean±SEM of triplicate assays performed at least three times.

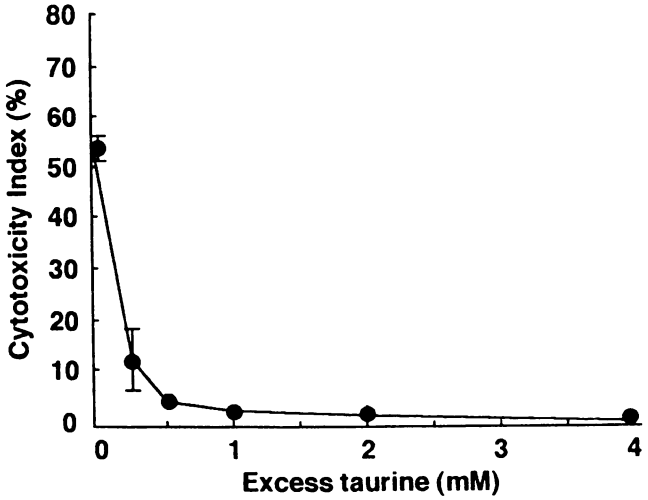

Figure 7. Protective effect of taurine added to $HOCl/OCl^-$ in a concentration exceeding that of $HOCl/OCl^-$. ^{51}Cr-labeled AKD cells were incubated 7 h at 37°C, with an equimolar concentration of taurine and $HOCl/OCl^-$, in addition to 0–4 mM taurine, referred to as "excess taurine" on the abscissa. The CI was determined as described in the text. Results represent the mean±SEM of four determinations performed in triplicate.

toxicity than the AKD cell line (Fig. 11 *B*). None of the transfected cell lines expressing either wild-type CFTR (NP-13.21) or mutant ΔF508 CFTR (NP-56.8, NP-56.12, and NP-56.13) showed an increase in susceptibility to taurochloramine injury when compared to NP-34, and only the NP-56.12 was significantly less susceptible to taurine chloramine cytotoxicity (NP-56-12 vs. NP-34: $P < 0.01$ at 2, 3, and 4 mM taurine chloramine). As with the AKD cell line, 100 μM DIDS, and 100 μM DIDS in chloride-free buffer, suppressed taurine chloramine–mediated cytotoxicity in all NP cell lines (data not shown).

Discussion

High concentrations of the amino acid, taurine, were observed in the airway secretions of patients with either cystic fibrosis or other inflammatory lung diseases known to be associated with a neutrophil influx to the airways. Most of the taurine observed in CF sputum was extracellular. The source of the taurine remains unknown, but likely includes neutrophils, cells with a high taurine concentration (5). A strong inverse correlation was observed between CF sputum taurine and the number of days of intravenous antibiotic therapy, suggesting that taurine levels reflect the degree of airway inflammation. Taurine reacts readily with myeloperoxidase-derived $HOCl/OCl^-$ to form long-lived anionic oxidants known as taurochloramines (8). No chloramines were detected in vitro in CF sputum. Taurochloramines are highly reactive oxidants that react rapidly with many molecules, such as thiols, proteins, and lipids. In addition, as reported in this study, taurochloramines can be taken up by epithelial cells. Each of these characteristics may contribute to shortening the half-life of taurochloramines in respiratory secretions. The absence of detectable amounts of taurochloramines in CF sputum therefore does not exclude mono- and dichloramine synthesis in vivo.

Table I. Uptake of [^{3}H]Taurine Chloramine by Lung Epithelial Cells

Conditions	dpm	% control	*P* value‡
Control (HPBR)	21,448±787*	100	—
DIDS 100 μM	16,898±612	79	$P < 0.05$
Chloride-free	15,525±2,018	72	$P < 0.05$
DIDS 100 μM + Cl-free	7,311±1,477	34	$P < 0.001$
Cold (4°C)	8,438±814	39	$P < 0.001$

* Values represent the mean±SEM of triplicate assays performed on five separate occasions. ‡ Comparisons are made with control.

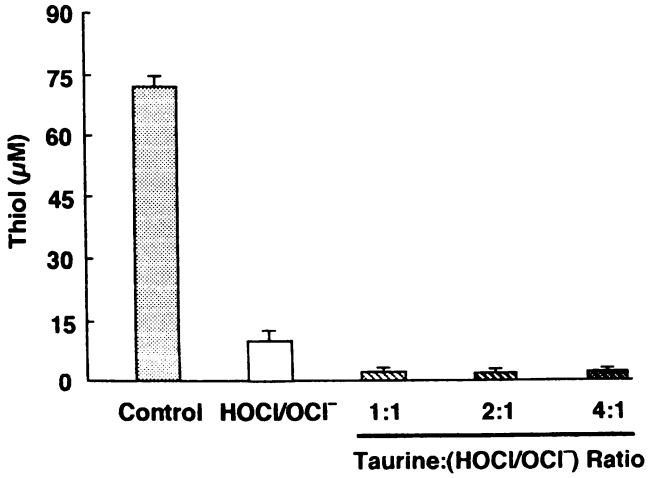

Figure 8. Effect of taurine on $HOCl/OCl^-$-mediated oxidation of thiol in vitro. Hypochlorous acid, 2 mM, and 0–8 mM taurine were incubated with a solution of 80 μM reduced DTNB. Thiol oxidation was measured after a 15-min incubation at 25°C, using a spectrophotometer at 412 nm, and assuming $\Sigma_M = 13{,}600$.

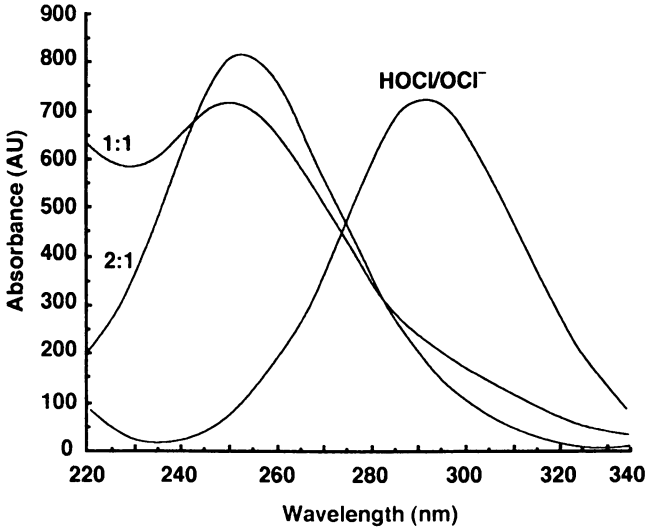

Figure 9. Modification of the spectral properties of $HOCl/OCl^-$ by different concentrations of taurine. The characteristic maximal absorbance peak of $HOCl/OCl^-$ was observed at 292 nm. With the addition of an equimolar concentration of taurine to $HOCl/OCl^-$, the wavelength of the maximal absorbance peak shifted to 252 nm. A 2:1 molar excess of taurine/$HOCl/OCl^-$ resulted in a decrease of absorbance at 252 nm and an increase in the absorbance at the wavelength of 220 nm. Shown is the result of a typical experiment representative of results obtained in five separate determinations.

Cystic fibrosis sputum has a high concentration of myeloperoxidase, which can synthesize $HOCl/OCl^-$ and induce respiratory epithelial cell cytotoxicity (7). This study demonstrates that taurine not only increases the amount of $HOCl/OCl^-$ needed to induce lung epithelial cell cytotoxicity in vitro, but also changes the mechanisms by which lung cell injury occurs. In contrast to $HOCl/OCl^-$–mediated injury, taurine

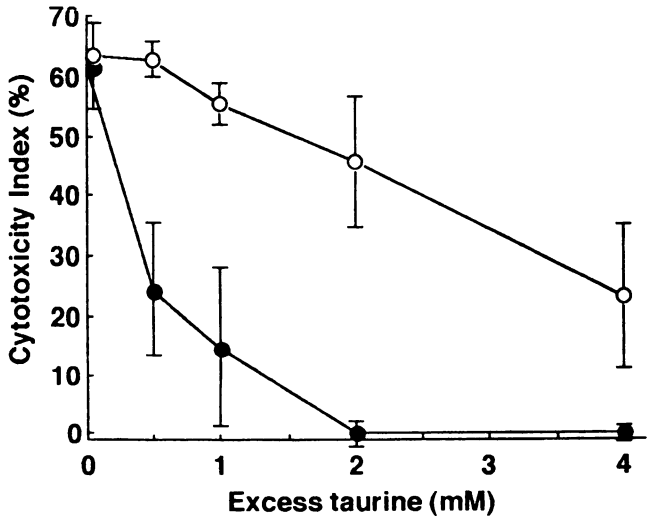

Figure 10. Effect of bicarbonate on the protective properties of taurine against $HOCl/OCl^-$–mediated cytotoxicity in AKD cells. Experiments were performed as described in Fig. 7, using EBSS media containing either high (●, 26 mM, pH 7.4) or low (○, 2.6 mM, pH 6.4) sodium bicarbonate concentrations. Much higher concentrations of taurine were needed at low bicarbonate levels to protect the cells against an identical burden of $HOCl/OCl^-$.

chloramine–mediated lung epithelial cell injury is dependent upon cellular uptake of oxidants through anion transport systems. Anion entry into epithelial cells may occur either through electrically neutral mechanisms, such as anion exchange, or through conductance channels in which anion cell entry generates changes in the electrical potential difference across the cell membrane (25).

Several lines of evidence suggest that taurine transforms $HOCl/OCl^-$ to oxidant organic anions, which are toxic to lung epithelial cells if they are taken up by the cells through anion exchange mechanisms. First, DMSO, a potent $HOCl/OCl^-$ scavenger, provided complete protection against $HOCl/OCl^-$ alone but did not prevent either cytotoxicity or thiol oxidation in the presence of an equimolar concentration of taurine. Since chloramines do not react with DMSO (18), these results are consistent with the formation of taurine chloramines. Taurine also shifted the maximal absorption of $HOCl/OCl^-$ from 291 nm to wavelengths characteristic of taurine monochloramines at 252 nm and taurine dichloramines at less than 220 nm (18). Second, while DIDS was unable to provide significant protection against $HOCl/OCl^-$ alone, DIDS was fully protective in the presence of an equimolar concentration of $HOCl/OCl^-$ and taurine. Furthermore, the protective effect of DIDS in the presence of $HOCl/OCl^-$ and taurine persisted after extensive washing of the DIDS-treated cells, suggesting that it was the DIDS tightly bound to the cells, which was responsible for the protective effect. Since DIDS is known to covalently bind and inhibit the anion exchanger (26, 27), these results are consistent with the concept that taurine chloramine, but not $HOCl/OCl^-$–induced lung epithelial cell cytotoxicity, was dependent upon uptake of the toxic oxidants through an anion exchange system. Third, although reduction of the incubation temperature to 4°C, a condition known to markedly slow anion exchange (28), did not protect lung epithelial cells against $HOCl/OCl^-$, it provided complete protection against $HOCl/OCl^-$ in the presence of taurine, again suggesting that taurine chloramine but not $HOCl/OCl^-$–mediated cytotoxicity is dependent upon anion exchange. Fourth, incubation of the lung epithelial cells in chloride-free buffer, a condition likely to lead to a reduction of anion exchange activity (29), resulted in partial protection against $HOCl/OCl^-$ and taurine. The protective effect of chloride-free buffer was further enhanced by the addition of DIDS. Finally, each of the conditions found to protect lung epithelial cells against HOCl/taurine, i.e., DIDS, cold temperature, chloride-free buffer, and chloride-free buffer with DIDS, resulted in a proportional reduction of taurine chloramine uptake by the lung epithelial cells.

The effect of taurine on lung epithelial cell $HOCl/OCl^-$–dependent cytotoxicity is similar to that described with red blood cells in which the anion exchanger has been shown to play a critical role in defining susceptibility to neutrophil-derived oxidant injury (11). One of the important features in defining lung epithelial cell susceptibility to taurine and $HOCl/OCl^-$–mediated cytotoxicity was the ratio of taurine to $HOCl/OCl^-$. In this study, cytotoxicity was evident at a 1:1 molar ratio of taurine to $HOCl/OCl^-$, but was suppressed by selectively increasing the taurine concentration. It is unlikely that the protective effect of a molar excess of taurine was related to protection of sulfhydryl groups, since high taurine to $HOCl/OCl^-$ ratios did not prevent the oxidation of thiol in vitro. Similar results demonstrating oxidation of α-1-proteinase inhibitor by $HOCl/OCl^-$ in the presence of high concentrations

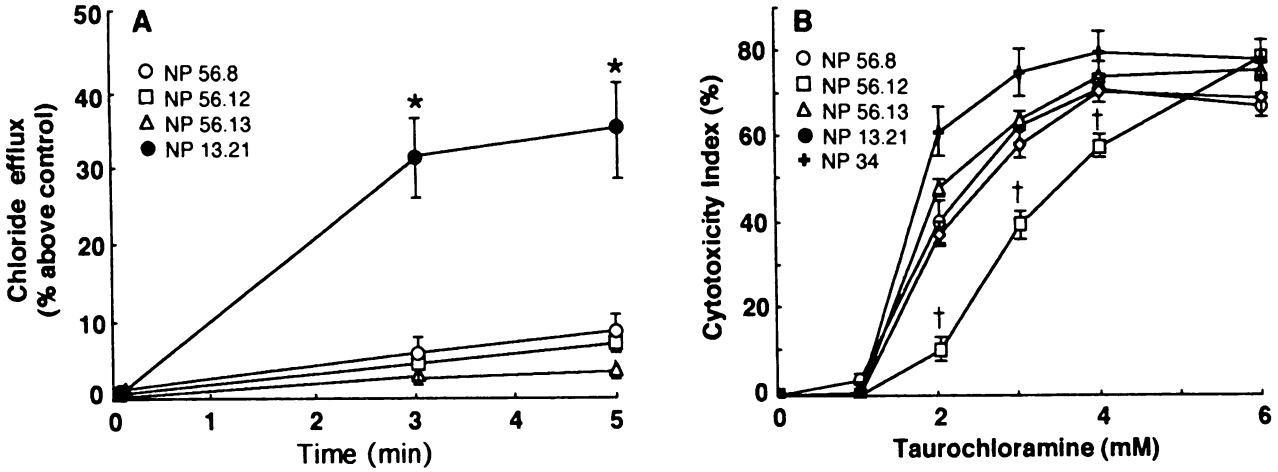

Figure 11. Comparison of (*A*) chloride efflux ($n = 6$) and (*B*) susceptibility to taurochloramine-induced cytotoxicity in human nasal polyp SV-40 immortalized cells, which do not express CFTR cDNA (+) or which have been transfected with either wild-type CFTR cDNA (●) or ΔF508 mutant CFTR cDNA (△, □, ○). Results of cytotoxicity represent the mean±SEM of triplicate assays performed four times. $^{*}P < 0.01$NP-13.21 vs. NP-56.8, NP-56.12, and NP-56.13. $^{\dagger}P < 0.01$ NP-56.12 vs. NP-34.

of taurine (10), suggest that, while taurine can protect epithelial cells against $HOCl/OCl^-$–mediated injury, it does not decrease the oxidizing potential of $HOCl/OCl^-$ towards molecules in the extracellular milieu. The mechanism by which a molar excess of taurine protects lung epithelial cells is likely related to the transformation of highly toxic taurine dichloramines to monochloramines. Taurine dichloramine is a much more efficient chlorinating agent of cell components, such as tyrosine residues, than is taurine monochloramine (11). Furthermore, the R moiety rather than the Cl^- atom of dichloramines ($RNCl_2$), but not monochloramines (RNHCl), can be incorporated into acceptor molecules (30). Evidence that an excess molar ratio of taurine favored the formation of less toxic monochloramines is provided by the observation that increasing concentrations of taurine induced an increase in absorption at 252 nm and a decrease in absorbance at 220 nm, as would be expected for monochloramines. This study suggests that, as observed in red blood cells (11), taurine may prevent $HOCl/OCl^-$–mediated lung epithelial cell lysis by decreasing chlorination of cell components rather than by preventing oxidation of sulfhydryl groups.

The balance between toxic taurine dichloramines and the less toxic monochloramines is not only dependent upon the taurine to $HOCl/OCl^-$ ratio, but also upon pH. Acidification is known to increase the amount of taurine needed to convert toxic taurine dichloramines to the much less toxic taurine monochloramines according to the following reaction:

$$\text{Taurine} + \text{TauNCl}_2 \underset{\downarrow pH}{\overset{\uparrow pH}{\rightleftarrows}} 2\text{TauNHCl} + H_2O.$$

In this context, acidification of the culture medium should increase the concentration at which taurine will provide lung epithelial cell protection against $HOCl/OCl^-$. In this study, although reducing the culture medium pH to 6.4 did not in itself create cytotoxicity, we observed, at pH 6.4, a marked increase in the amount of taurine needed to provide the same degree of antioxidant protection as observed at the more alkaline pH 7.4 (Fig. 10). Acidification of the medium resulted in a 12% decrease in cellular GSH, which may have contributed, at least in part, to the increased susceptibility of the cells to taurine chloramine–mediated injury. However, these results are also consistent with the concept that protection of lung epithelial cells by taurine against $HOCl/OCl^-$–dependent cytotoxicity may be mediated by the generation of the less cytotoxic taurine monochloramines.

The effect of extracellular bicarbonate concentration on lung epithelial cell susceptibility to neutrophil-derived oxidant injury may be particularly relevant to the lung in CF. The basic defect in CF is related to mutations of the gene that encodes the CFTR (31). Experimental evidence indicates that CFTR functions, at least in part, as a regulated anion channel (32). Approximately 70% of patients with CF have a deletion of a single amino acid within the first nucleotide binding fold at position 508 of the primary CFTR structure, leading to the trapping of CFTR within the endoplasmic reticulum and the absence of CFTR function in the apical membranes of affected epithelial cells (33, 34). Native respiratory epithelium has been shown to have a high capacity for bicarbonate secretion, but cAMP-induced bicarbonate secretion is absent in CF airway epithelial cells (35). The deficient bicarbonate secretion by the CF airway epithelium is thought to result in a significant degree of acidification of airway secretions, a situation that would favor the formation of the highly toxic taurine dichloramines. This study does not provide evidence that taurochloramines play a role in vivo in the CF lung. However, it is of interest that aminoglycosides, molecules capable of converting $HOCl/OCl^-$ to noncytotoxic chloramines, help preserve lung function when administered chronically to CF patients by inhalation (36–38).

The effect of taurine on $HOCl/OCl^-$–dependent cytotoxicity was not limited to the AKD cell line, since transformed human upper respiratory epithelial cells derived from nasal polyps were also susceptible to injury through a DIDS-inhibitable pathway. The expression of wild-type and mutant CFTR cDNA in these cell lines resulted in significant differences in chloride permeability, but did not change the susceptibility of the cell lines to taurochloramine injury. These results are consistent with the concept that taurochloramine uptake was mediated by anion exchange, a pathway that has recently been shown to be present in human bronchial epithelial cells and is

unaffected by the expression of either wild-type or mutant CFTR cDNA in respiratory epithelial cells (39, 40).

In summary, patients with CF and other inflammatory airway disorders, have high taurine concentrations in their respiratory secretions. The levels of CF sputum taurine markedly decrease during antibiotic treatment of acute respiratory exacerbations. In vitro, taurine converts $HOCl/OCl^-$ to chloramines, which are less cytotoxic than $HOCl/OCl^-$, but once taken up by lung epithelial cells through anion transport mechanisms, can subsequently induce cytotoxicity. Lung epithelial cell injury is decreased under conditions that favor taurine monochlor-amine formation, such as an excess molar ratio of taurine to $HOCl/OCl^-$, and an alkaline pH. These results suggest that taurine plays a role in protecting lung epithelial cells against myeloperoxidase-derived oxidants. However, the efficacy of this protective mechanism may be significantly decreased in an acidified milieu.

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