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Am J Physiol Lung Cell Mol Physiol 293:L1156-L1162, 2007. First published 24 August 2007;
doi:10.1152/ajplung.00081.2007

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Am J Physiol Lung Cell Mol Physiol 293: L1156–L1162, 2007.
First published August 24, 2007; doi:10.1152/ajplung.00081.2007.

Cigarette smoke irreversibly modifies glutathione in airway epithelial cells

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Submitted 5 March 2007; accepted in final form 16 August 2007

Van der Toorn M, Smit-de Vries MP, Slebos D-J, de Bruin HG, Abello N, van Oosterhout AJ, Bischoff R, Kauffman HF. Cigarette smoke irreversibly modifies glutathione in airway epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 293: L1156–L1162, 2007. First published August 24, 2007; doi:10.1152/ajplung.00081.2007.—In patients with chronic obstructive pulmonary disease (COPD), an imbalance between oxidants and antioxidants is acknowledged to result in disease development and progression. Cigarette smoke (CS) is known to deplete total glutathione (GSH + GSSG) in the airways. We hypothesized that components in the gaseous phase of CS may irreversibly react with GSH to form GSH derivatives that cannot be reduced (GSX), thereby causing this depletion. To understand this phenomenon, we investigated the effect of CS on GSH metabolism and identified the actual GSX compounds. CS and H_2O_2 (control) deplete reduced GSH in solution [$\Delta = -54.1 \pm 1.7$ μM ($P < 0.01$) and -39.8 ± 0.9 μM ($P < 0.01$), respectively]. However, a significant decrease of GSH + GSSG was observed after CS ($\Delta = -75.1 \pm 7.6$ μM, $P < 0.01$), but not after H_2O_2. Exposure of A549 cells and primary bronchial epithelial cells to CS decreased free sulfhydryl (-SH) groups ($\Delta = -64.2 \pm 14.6$ μM/mg protein, $P < 0.05$) and irreversibly modified GSH + GSSG ($\Delta = -17.7 \pm 1.9$ μM, $P < 0.01$) compared with nonexposed cells or H_2O_2 control. Mass spectrometry (MS) showed that GSH was modified to glutathione-aldehyde derivatives. Further MS identification showed that GSH was bound to acrolein and crotonaldehyde and another, yet to be identified, structure. Our data show that CS does not oxidize GSH to GSSG but, rather, reacts to nonreducible glutathione-aldehyde derivatives, thereby depleting the total available GSH pool.

chronic obstructive pulmonary disease; mass spectrometry; aldehydes; acrolein; crotonaldehyde

SMOKING AND GENETIC SUSCEPTIBILITY are the main risk factors for the development of chronic obstructive pulmonary disease (COPD) (2, 22). One important hypothesis with respect to the pathophysiology of COPD is an imbalance between oxidants and antioxidants in the airways. This imbalance results in disease when the antioxidant capacity of the lung is unable to sufficiently neutralize reactive compounds present in cigarette smoke (CS) or generated during the persistent airway inflammation in COPD (18).

Reactive compounds in the gaseous phase of CS are thought to constitute the main component of the oxidative stress in COPD (15). In patients with COPD, increased levels of oxidative stress parameters have been documented in exhaled breath condensate, sputum, and blood: higher H_2O_2, 8-isoprostane, and malondialdehyde levels and lower reduced glutathione (GSH) levels (3, 11). Changes induced by these reactive components may result in inactivation of antiproteases, epithelial cell injury, apoptotic and necrotic cell death, mitochondrial dysfunction, disturbance of extracellular matrix repair, and maintenance of airway inflammation, all potentially of importance in the development of COPD (25, 32).

Since the airways of smokers are exposed to highly reactive components, the lung is always at risk of oxidative injury (26). To ensure an appropriate defense against this injury, the respiratory tract is equipped with the epithelial lining fluid (ELF) and the airway epithelium, which contain large amounts of GSH (9). GSH plays a key role in the cellular redox balance and is thought to be one of the most important antioxidant defenses against inhaled reactive components of CS (4).

Under nonstress conditions, most of the intracellular GSH is "stored" in its reduced form. However, the balance between GSH and oxidized glutathione (GSSG) can change significantly under conditions of oxidative stress, and the ratio of GSH to GSSG provides information on the redox status of cells and tissues. During increased oxidative stress, the free sulfhydryl (-SH) groups become oxidized (33). It has been described that exposure to the gaseous phase of CS in vitro and in vivo generally results in a loss of GSH, whereas the amount of GSSG does not increase significantly (21, 26). This was confirmed by preliminary experiments performed in our laboratory: we observed an irreversible loss of total glutathione in epithelial cells exposed to the gaseous phase of CS.

We therefore hypothesized that components of the gaseous phase of CS may irreversibly react with GSH to form GSH derivatives that cannot be reduced (GSX). Consequently, GSX may induce a chronic lack of protection against additional CS exposure when de novo synthesis of GSH is rate limiting. This may occur when there is a genetic deficiency in de novo synthesis of GSH that predisposes smokers to the development of COPD (8, 14).

MATERIALS AND METHODS

Chemicals. L-Cysteine, BSA, GSH, GSSG, H_2O_2, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), trichloroacetic acid (TCA), acrolein, and crotonaldehyde were obtained from Sigma-Aldrich Chemie (Zwijndrecht, The Netherlands).

Cell cultures. The human alveolar type II epithelium-like adherent cell line A549 was purchased from American Type Culture Collection (Manassas, VA). Human primary bronchial epithelial cells were collected and cultured as described previously (32). All cells were

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grown in RPMI 1640 medium with 25 mM HEPES, L-glutamine (BioWitthaker, Verviers, Belgium) supplemented with 10% heat-inactivated fetal calf serum (BioWitthaker), and 20 μg/ml gentamicin (Centafarm Services, Etten-Leur, The Netherlands). All cells were grown in 25-cm² plastic culture flasks (Costar, Cambridge, MA) at 37°C in an atmosphere of 5% CO_2 until 80–90% confluency was reached. Before the experiments, A549 cells and human primary bronchial epithelial cells were incubated for 16 h in serum-free RPMI 1640 medium.

Exposure of GSH solution to different oxidants. A solution of GSH (150 μM in pure H_2O, pH 4.2) was exposed to air, the gaseous phase of one cigarette (CS) or two cigarettes [CS(2×)], or a solution of 1 mM H_2O_2. Briefly, the solution (25 ml) was placed in a 50-ml Falcon tube (BD Biosciences, Alphen aan den Rijn, The Netherlands) at 37°C. Kentucky 2R4F research-reference cigarettes (Tobacco Research Institute, University of Kentucky, Lexington, KY) were smoked using a peristaltic pump. Just before the experiments, filters were cut from the cigarettes. Each cigarette was smoked in exactly 5 min at a flow rate of 8 l/h and bubbled through the GSH solution. This solution was used immediately for the experimental procedures. Air produced with the same peristaltic pump, but without the cigarette, was used as a negative control under the same conditions. The oxidative agent H_2O_2 (1 mM final concentration) was used as a positive control.

Exposure of airway epithelial cells to CS. A549 cells were exposed to air, CS, or a solution of 5 mM H_2O_2. Briefly, A549 cells were grown in 25-cm² plastic culture flasks as described above. Just before the experiments, medium was removed and the culture flask was positioned upside down, allowing direct contact of smoke with epithelial cells. Kentucky 2R4F research-reference cigarettes were smoked using a peristaltic pump. Just before the experiments, filters were cut from the cigarettes. Each cigarette was smoked in exactly 5 min at a rate of 8 l/h. The smoke inside was blown through a small plastic tube for direct distribution of the gaseous phase of CS inside the culture flasks. After the exposure, cells were washed with PBS, lysed with water, and analyzed. Air was used as negative control under the same conditions used for CS. As positive control, the cells were incubated in H_2O_2 (5 mM final concentration) at room temperature for 5 min and then washed with PBS, lysed with water, and analyzed.

Quantitative determination of total -SH groups. A549 cells were washed with PBS and lysed by one freeze-thaw cycle in 2.5 ml of pure H_2O. Total protein concentration was determined by the Bradford method (7), with BSA (Bio-Rad Laboratories) used as a standard. Ellman's reagent (12) was used for the determination of free -SH groups in cell culture and a cell-free solution of GSH. Ellman's reagent (12 mM DTNB) was added to the lysed cells or GSH solution to a final concentration of 6 mM DTNB, and the sample was incubated for 10 min. Thereafter, the samples were centrifuged at 1,000 *g* for 5 min. The supernatant was used in the assay and measured at 405 nm in a microplate reader (model EL808, Bio-Tek Instruments, Abcoude, The Netherlands). The amount of free thiol groups was calibrated against a standard curve of L-cysteine.

Quantitative determination of GSH + GSSG using the Tietze assay. GSH + GSSG in different samples was determined by the enzymatic method of Tietze (31). Briefly, cells were washed with PBS and treated with 5% TCA. The samples of the GSH solution were also treated with 5% TCA. All samples were centrifuged at 10,000 *g* for 5 min at 4°C. Then 150 μl of each standard and sample were pipetted into a 96-well ELISA plate, and 25 μl of Ellman's reagent (DTNB) and 5 μl of gluthatione reductase (GR) were added. This supernatant was enzymatically reduced by the added GR, and immediately before absorbance was read, NADPH was added and the increase of absorbance at 405 nm was recorded for 10 min at room temperature on a microplate reader (model EL808, Bio-Tek). The results were compared with a standard curve of GSH.

Mass spectrometry. Before analysis of A549 cell lysates, primary bronchial epithelial cell lysates, and solutions of GSH and GSSG by mass spectrometry (MS), samples were filtered using an Amicon Ultra 5-kDa-cutoff centrifugal filter device (Millipore, Cork, Ireland). The collected ultrafiltrate was diluted 1:1 with 0.1% (vol/vol) formic acid (Merck, Haarlem, The Netherlands) in acetonitrile (Merck). The samples were analyzed by direct infusion at 5 μl/min into an SL ion trap mass spectrometer (Agilent, Santa Clara, CA) equipped with an electrospray ionization source operated in positive mode at 275°C under the following conditions: capillary voltage was 3,000 V, nebulizer gas was N_2 at 10 psi, and drying gas was N_2 at 4 l/min. MS data were acquired over a scan range of 50–650 mass-to-charge ratio (*m/z*) and with a scan rate of *m/z* 5,500 per second. The target *m/z* was 615, and the compound stability was set at 70%. Data were collected for 10 min before mass spectrum averaging and analyzed using data analysis software for LC/MSD Trap version 3.2 (Bruker Daltonics, Bremen, Germany). Peaks of interest were fragmented using manual MS-MS (MS2) settings (width of precursor ion selection = 1.0–1.5 Da, amplitude = 0.8–1.0 V). MS2 data were collected for 5 min.

Statistical analysis. Data were analyzed using Prism 4 for Windows (GraphPad Software, San Diego, CA). Comparisons between different experimental groups were performed with Dunnett's multiple comparison test (Fig. 1; see Fig. 3). $P < 0.05$ was considered significant. Values are means ± SE unless otherwise mentioned.

RESULTS

CS decreases GSH + GSSG in solution. GSH is one of the most important antioxidants in the ELF and participates in the defense of pulmonary epithelial cells against inhaled reac-

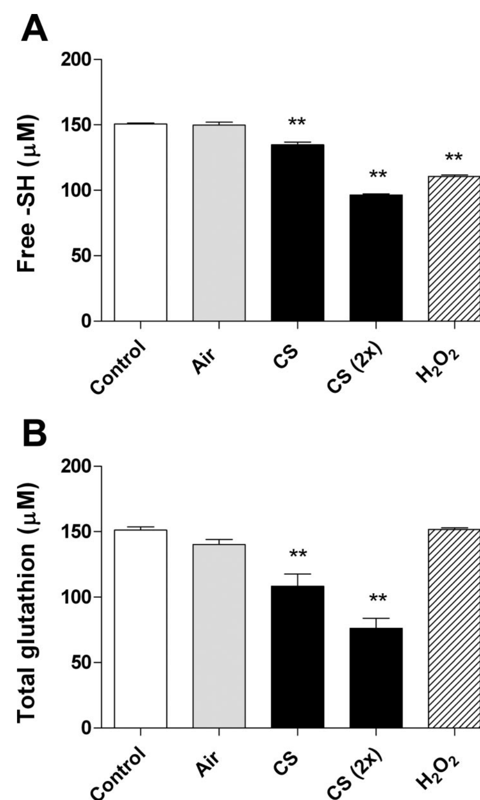

Fig. 1. Effect of cigarette smoke, control air, and H_2O_2 on the level of free sulfhydryl (-SH) groups of GSH in solution. *A*: free -SH groups studied using Ellman's reagent. *B*: total glutathione (GSH + GSSG) studied using the enzymatic reducing cycle system of Tietze. CS, exposure to 1 cigarette; CS(2×), exposure to 2 cigarettes. Values are means ± SE from 4 experiments. $^{**}P < 0.01$ vs. control (Dunnett's multiple comparison test).

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tive components of CS. To test whether these CS components are able to modify the free -SH groups of GSH, a solution of GSH was exposed to CS. Although air, used as negative control, did not affect the level of free -SH groups of GSH, exposure to CS(2×) (Δ = −54.1 ± 1.7 μM, $P < 0.01$; Fig. 1*A*) or addition of 1 mM H_2O_2 (Δ = −39.8 ± 0.9 μM, $P < 0.01$; Fig. 1*A*) resulted in a dose-dependent decrease of free -SH groups.

To determine whether CS oxidizes GSH to GSSG, the enzymatic reducing cycle of Tietze was used. A significant reduction of GSH + GSSG was observed after CS(2×) (Δ = −75.1 ± 7.6 μM, $P < 0.01$; Fig. 1*B*), whereas H_2O_2, which has been proven to oxidize GSH (Fig. 1*A*), did not affect GSH + GSSG concentration compared with control (Fig. 1*B*).

MS analysis of CS-exposed GSH in solution. To understand why CS decreases free -SH groups in solution, samples were analyzed by MS via direct infusion. Figure 2, *A* and *B*, shows the MS background of pure water and water exposed to CS(2×). Water exposed to CS shows a substantial background between *m/z* 80 and 300. Because the background was less prominent above *m/z* 300, we were able to study glutathione modification (*m/z* 308.1, singly charged protonated GSH; Fig. 2*C*). In Fig. 2*D*, two peaks corresponding to GSSG can be seen. The peak with *m/z* 307.1 indicates double-charged GSSG, and the peak with *m/z* 613.2 indicates single-charged GSSG. MS analysis of GSH in solution exposed to CS(2×) did not result in the formation of oxidized GSSG. However, two additional components (*components 1* and *2*) were observed with *m/z* 364.1 and 378.1, respectively (Fig. 2*E*). Addition of H_2O_2 to GSH in solution, as the control experiment, resulted in two peaks of *m/z* 307.2 and 613.2, proving the direct oxidation of GSH to GSSG (Fig. 2*F*).

CS decreases total -SH groups in airway epithelial cells. To investigate the effect of CS on the redox status of free -SH groups, airway epithelial cells were directly exposed to CS. Air exposure used as a negative control did not affect free -SH groups in the cells, whereas the gaseous phase of CS significantly decreased this level by −64.2 ± 14.6 μM/mg protein ($P < 0.05$; Fig. 3*A*). No effect was observed when H_2O_2 was used as positive control, perhaps because of the short incubation period (30).

CS decreases GSH + GSSG in airway epithelial cells. To examine whether glutathione was irreversibly modified after CS exposure, GSH + GSSG was determined using the enzymatic GSSG-reducing cycle system described by Tietze (31). A significant reduction of GSH + GSSG was observed after the A549 cells were exposed to CS (Δ −17.7 ± 1.9 μM, $P < 0.01$; Fig. 3*B*). Exposure to air and H_2O_2 did not affect the GSH + GSSG concentration in the epithelial cells compared with control cells.

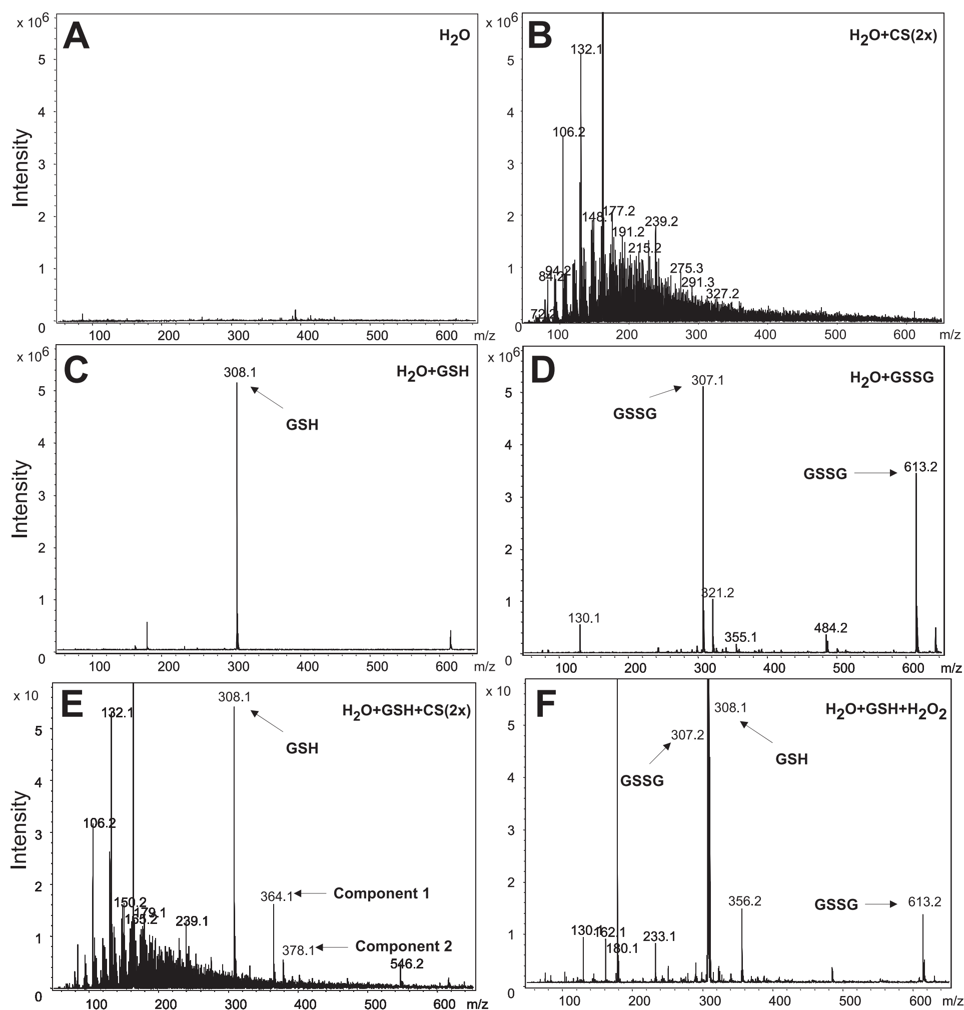

Fig. 2. Effect of CS and H_2O_2 on modification of a cell-free GSH solution by mass spectrometry (MS). *A*: sample solution (pure H_2O). *B*: H_2O + CS(2×). *C*: H_2O + GSH (150 μM final concentration). *D*: H_2O + GSSG (150 μM final concentration). *E*: H_2O + GSH + CS(2×). *F*: H_2O + GSH + H_2O_2 (1 mM final concentration). Mass spectra are representative of 1 of 3 experiments.

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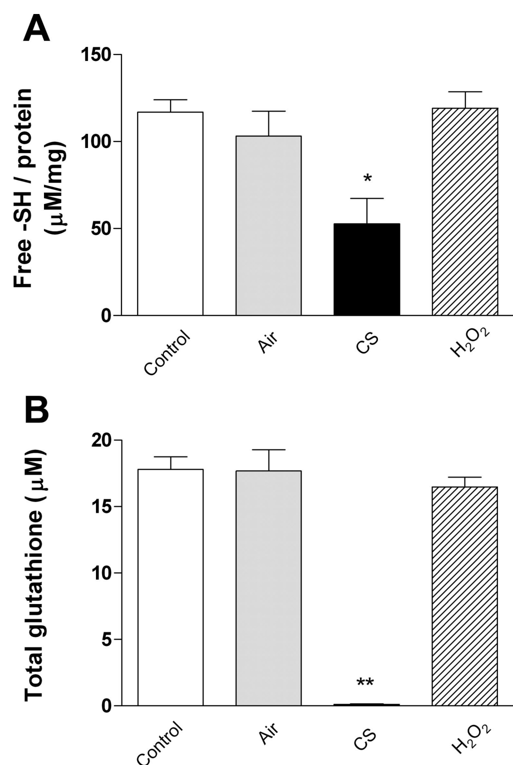

Fig. 3. Effect of CS on free -SH groups in A549 cells. *A*: measurement of free -SH groups in A549 cells using Ellman's reagent. *B*: examination of irreversible modification of glutathione after exposure of A549 cells to cigarette smoke by measurement of GSH + GSSG using the enzymatic reducing cycle system of Tietze. Values are means ± SE of 4 experiments. $*P < 0.05$; $**P < 0.01$ vs. control (Dunnett's multiple comparison test).

MS analysis of airway epithelial cells. A549 cells were exposed directly to the gaseous phase of CS and then lysed. The lysate was directly analyzed by MS via direct infusion. Nonexposed A549 cells (Fig. 4*A*) and cells exposed to air (Fig. 4*B*) show a peak at *m/z* 308.1, corresponding to the reduced form of GSH. We observed a decreased signal for GSH in the CS-exposed cells (Fig. 4*C*) compared with the untreated or air-exposed cells, as well as three different components (*components 1, 2*, and *3*). *Components 1* and *2* were also observed in the CS-exposed GSH solution: peaks at *m/z* 364.1 (*component 1*) and 378.1 (*component 2*). Furthermore, a new peak corresponding with *m/z* 391.2 (*component 3*) appeared. Short exposure of A549 cells to H_2O_2 did not alter GSH compared with the nonexposed cells (Fig. 4*D*). Interestingly, GSSG (peaks at *m/z* 307.1 and 613.2) was not detectable in any of the A549 cultures.

MS showed GSH modification to glutathione-aldehyde derivatives. MS analysis of CS-exposed A549 cells resulted in a decreased peak at *m/z* 308.1, reduced GSH, and additional peaks at *m/z* 364.1 (*component 1*), 378.1 (*component 2*), and 391.2 (*component 3*). The isotope distribution confirmed that these *m/z* values corresponded to singly charged molecules. By subtracting the mass of these components from the mass of GSH, we were able to select a few candidate molecules, including acrolein and crotonaldehyde, that would coincide with the observed mass difference. After incubation of GSH with acrolein and crotonaldehyde, we were able to detect peaks at *m/z* 364.1 and 378.1, respectively (Fig. 5, *A* and *B*). These peaks were identified by MS2 fragmentation. The fragments obtained from the A549 cells exposed to the gaseous phase of CS were compared with GSH incubated with acrolein (GSH + acrolein) or crotonaldehyde (GSH + crotonaldehyde). These findings were also confirmed by MS2 fragmentation of primary bronchial epithelial cell lysates under the same experimental conditions used for the A549 cells (Fig. 5, *C* and *D*). Fragmentation confirmed the identity of the peaks to be GSH-acrolein and GSH-crotonaldehyde (*components 1* and *2*, respectively; Fig. 5, *C* and *D*). The same cell lysate also showed a peak at *m/z* 391.2 (*component 3*). We have not been able to identify this peak, apart from the fact that it did not derive from phthalate contamination. Phthalates, from plastic bottles, flasks, and packing materials, are well-known contaminants in

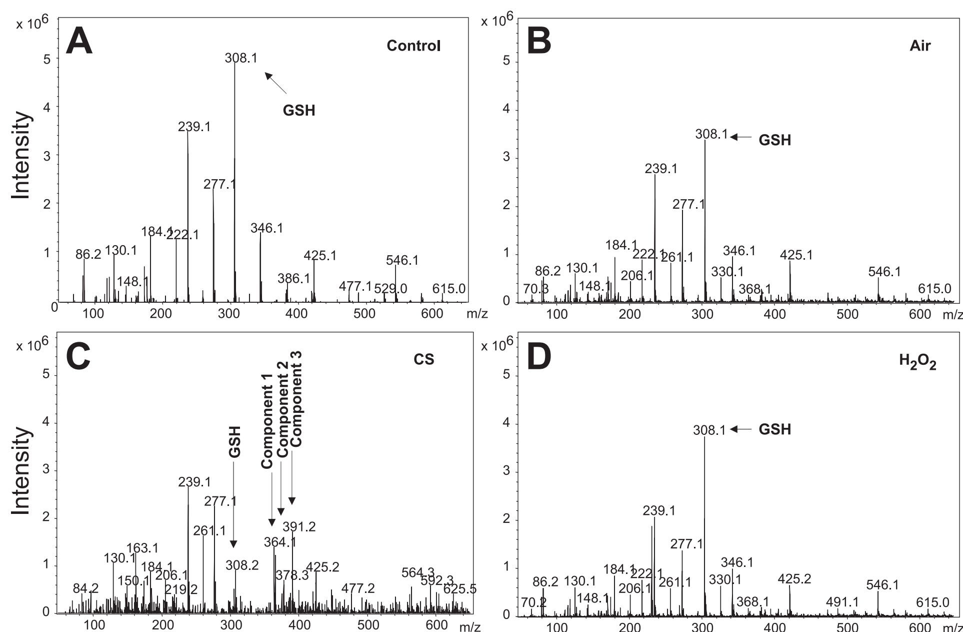

Fig. 4. Effect of CS and H_2O_2 on modification of GSH in A549 cells by MS. *A*: control (no exposure). *B*: air. *C*: CS. *D*: H_2O_2 (5 mM final concentration). Peaks at *m/z* 364.1 (*component 1*), 378.1 (*component 2*), and 391.2 (*component 3*) were characterized by MS-MS (MS2; see Fig. 5). Mass spectra are representative of 1 of 3 experiments.

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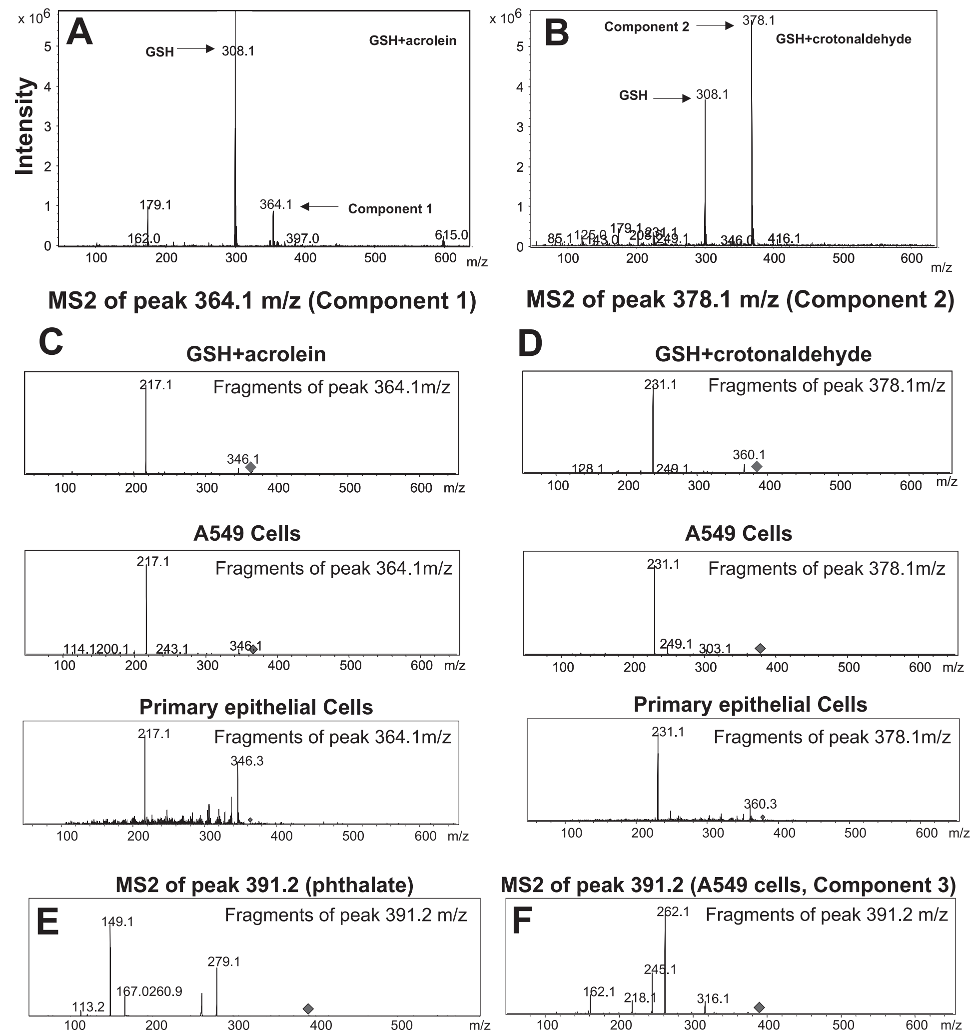

Fig. 5. Identification of GSH-acrolein (*m/z* 364.1, *component 1*) and GSH-crotonaldehyde (*m/z* 378.1, *component 2*) derivatives by MS2. *A*: mass spectra of GSH + acrolein. *B*: mass spectra of GSH + crotonaldehyde. *C*: 1st-daughter ion spectra of GSH + acrolein, A549 cells, and primary bronchial epithelial cells. *D*: 1st-daughter ion spectra of GSH + crotonaldehyde, A549 cells, and primary bronchial epithelial cells. *E*: 1st-daughter spectra of phthalate. *F*: 1st-daughter spectrum of the unknown component from A549 cells (*component 3*). Mass spectra are representative of 1 of 3 experiments.

MS (13). MS2 fragmentation of peak *m/z* 391.2 (*component 3*) obtained from traces of phthalate contamination differs from the MS2 fragmentation of peak *m/z* 391.2 obtained from the cell lysate (Fig. 5, *E* and *F*).

DISCUSSION

We have investigated the modification of GSH and, more generally, the total reduced thiol content of airway epithelial cells by the gaseous phase of CS. We hypothesized that CS can irreversibly modify GSH, rendering it unavailable for the enzymatic reducing cycle system, which is thought to play an important role in protection of airway epithelial cells against oxidative stress. Our results clearly showed that the gaseous phase of CS decreases free -SH groups of GSH in solution and in airway epithelial cells. We observed that the reactive components of the gaseous phase of CS did not oxidize GSH to the disulfide-containing GSSG. Instead of this physiological reaction, GSH was irreversibly modified by unsaturated aldehydes that are generated during the combustion of tobacco. In vitro experiments showed that exposure of CS changed almost the entire pool of GSH to GSH-aldehyde components.

These findings shed new light on the GSH redox cycle in airway epithelial cells. The enzymatic redox cycle, which is normally activated after oxidative stress and the formation of GSSG, could not be activated because of the depletion of GSH into nonreducible glutathione components, with loss of the GSH pool. This exhaustion of the pool of reduced GSH may induce a chronic lack of antioxidant protection. Persistent smokers may, in that case, inhale more ROS than can be scavenged by the residual antioxidants, resulting in increased vulnerability to oxidative stress. This makes the synthesis of GSH essential for cellular survival and protection of the lung.

The development of COPD is associated with increased oxidative stress and reduced antioxidant resources (5, 6, 18). Cigarette smoking is the most important factor for the development of COPD, which is the fifth leading cause of death worldwide (19). Cellular stress induced by CS is critically dependent on the intracellular reduced GSH concentration. For instance, intracellular GSH depletion significantly facilitates stress signal transduction pathways, cell proliferation, apoptosis, and inflammation (1). Rahman et al. (23, 27) showed that a GSH-to-GSSG ratio $<$90% influences a variety of cellular signaling processes, such as phosphorylation of stress kinases JNK, p38, MAPK, and phosphatidylinositol 3-kinase, as well as activation of the transcription factors activator protein-1 and NF-κB. They demonstrated that increasing the intracellular levels of GSH can provide protection. In other respiratory diseases such as cystic fibrosis, a significant decrease of

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GSH efflux from cells, which leads to deficiency of GSH in the ELF of the lung, as well as in other compartments, including cells of the immune system and the gastrointestinal tract, was observed (16).

Although reduced GSH has been shown to be elevated in the ELF of chronic smokers, CS acutely lowers intracellular levels of GSH (17, 24). An animal study by Cotgreave et al. (10) showed that acute effects of CS inhalation by rats caused significant depletion of GSH in the whole lung, lavage cells, and lavage fluid. The depleted GSH could not be reduced by a reducing agent such as dithiothreitol. They suggested that GSH was irreversibly conjugated with electrophilic components of the CS (10). Consistent with these results, intratracheal instillation of CS condensate into the rat resulted in depletion of intracellular GSH and concomitant formation of GSH conjugates without significant elevation of GSSG or any GSH efflux from the cells (24). These studies are in agreement with our current findings. We showed in a GSH solution that GSH exposed to the gaseous phase of CS was not oxidized to GSSG but, rather, was irreversibly modified to glutathione derivatives (Figs. 1 and 2). Where addition of the enzyme GR to a solution of GSH exposed to H_2O_2 resulted in a restoration of the initial GSH concentration, this enzyme was not able to reduce the components in the GSH solution exposed to CS, indicating that GSH had been irreversibly modified. However, this enzyme was not able to reduce the components in the GSH solution exposed to CS, indicating that GSH had been irreversibly modified. Exposure of cultured alveolar A549 cells to the gaseous phase of CS also showed that GSH was irreversibly modified to the same GSH derivatives, whereas incubation with H_2O_2 for 5 min did not alter the intracellular pool of GSH.

MS showed that the depletion of GSH could be attributed to the formation of glutathione-aldehyde derivatives. Direct infusion of cell lysate into the mass spectrometer showed three peaks (*m/z* 364.1, 378.1, and 391.2), whereas the peak of GSH (*m/z* 308.1) was diminished. We confirmed by MS2 that two of the three peaks were GSH-aldehyde derivatives: GSH-acrolein (*m/z* 364.1) and GSH-crotonaldehyde (*m/z* 378.1). Consistent with our findings, Reddy et al. (29) recently demonstrated that exposure of neutrophils to the gaseous phase of CS also resulted in the formation of GSH-acrolein. In contrast to our observations, Reddy et al. were not able to show GSH-aldehyde derivatives in airway epithelial cells. This may be due to *1*) a lower exposure to CS, *2*) a longer incubation period, allowing the release of the toxic components into the culture medium, or *3*) the use of cell culture medium, resulting in no direct contact of smoke with the cultured epithelial cells. In our system, medium was removed from the cells, and only a very thin layer of medium covered the cells, allowing a more direct contact of the gaseous phase of CS with the epithelial cells. After the exposure, cells were rapidly lysed and analyzed by MS via direct infusion. EC_{50} values of a variety of aldehydes that are able to deplete GSH showed that acrolein was by far the most effective one to bind to GSH followed by formaldehyde and crotonaldehyde (20). In contrast to our data from airway epithelial cells, the third peak (*m/z* 391.2) was not seen within the CS-exposed GSH solution. This peak is similar to a well-known contaminant in MS from phthalate compounds in plastic; however, fragmentation of the peak by MS2 showed that it was not a phthalate compound (13). It may be proposed that this component is derived from membrane degradation by radicals in CS, but the mass of this component could not be ascribed to known membrane degradation products.

Oxidative stress and decreased antioxidant capacity have important consequences for the pathogenesis of COPD. Activation of redox-sensitive transcription factors enhances proinflammatory mediators and protective antioxidant gene expression. In biochemistry, de novo synthesis of GSH from glutamate, cysteine, and glycine is catalyzed sequentially by the two cytosolic enzymes γ-glutamylcysteine and glutathione synthase. It is known that epithelial cells of the lung respond rapidly and sensitively to oxidative stress and that this adaptive response is mediated by an increase of γ-glutamylscysteine mRNA and enzyme activity (28). Although CS is the main environmental risk factor for developing COPD, only ~15–20% of smokers develop COPD. Genetic factors are likely to modify the risk of developing COPD. It can therefore be hypothesized that restricted ability to synthesize sufficient GSH may be due to polymorphisms in genes linked to de novo synthesis of GSH leading to COPD. A few studies are available involving polymorphisms in genes related to GSH synthesis and lung disease (8, 14). Therefore, further research on the variability among genes encoding for de novo GSH synthesis in smokers would be of great interest in human genetics of COPD.

For the first time, we have demonstrated by direct-infusion MS combined with enzymatic assays that a substantial amount of GSH in epithelial cells is irreversibly modified to GSH-acrolein and GSH-crotonaldehyde derivatives and a yet to be identified component. Under these circumstances, a chronic lack of protection against oxidative stress might be induced, especially when a genetic predisposition of rate-limiting de novo synthesis of GSH is present. These findings might be a biochemical mechanism of CS-induced toxicity that has been found in patients with COPD.

GRANTS

This work was supported by research grants of the J. K. de Cock Foundation (M. van der Toorn and D.-J. Slebos) and GlaxoSmithKline (H. G. de Bruin). N. Abello is the recipient of an Ubbo Emmius Fellowship from the University of Groningen.

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