

EDITORIAL

Oxidative stress and adaptive response of glutathione in bronchial epithelial cells

An important effect of oxidative stress and inflammation is the up-regulation of protective anti-oxidant genes. Among anti-oxidants, glutathione (GSH) and its redox enzymes appear to have an important protective role in the airspaces and intracellularly in epithelial cells where it is present in high concentrations. GSH has been implicated in numerous cellular functions, e.g. immune modulation, inflammatory responses, modulation of redox-regulated signal transduction, regulation of cell proliferation, remodelling of extracellular matrix, maintenance of surfactant and anti-protease screen, apoptosis, remodeling of extracellular matrix and mitochondrial respiration [reviewed in 1]. GSH has been shown to be critical to the lungs' anti-oxidant defences, particularly in protecting airspace epithelium (membrane integrity) from oxidant/free radical (cigarette smoke/air particulate)-mediated injury and inflammation [2, 3]. Alterations in the levels of reduced GSH in the lung lining fluid have been shown in various inflammatory conditions such as asthma, idiopathic pulmonary fibrosis and adult respiratory distress syndrome (ARDS) [reviewed in 1].

The synthesis of GSH requires the presence of two enzymes and the amino acids, glycine, cysteine and glutamate with cysteine being the rate-limiting substrate. The tripeptide GSH is formed by the consecutive actions of γ -glutamylcysteine synthetase (γ -GCS) and glutathione synthetase [4]. The rate-limiting enzyme in GSH synthesis is γ -GCS. The human γ -GCS heavy and light subunits are regulated by the transcription factor AP-1 and anti-oxidant response elements (ARE) and are modulated by oxidants, growth factors, inflammatory and anti-inflammatory agents in lung cells. In general, the activity of γ -GCS determines the rate of GSH synthesis. The reaction, catalysed by γ -GCS is feedback-inhibited by GSH [4]. The mammalian γ -GCS holoenzyme is a heterodimer consisting of a heavy subunit (γ -GCS-HS 73-kDa) and a light subunit (γ -GCS-LS 30-kDa) [5]. Although the heavy subunit contains all of the catalytic activity, γ -GCS activity can be modulated by the association of the heavy subunit with the regulatory light subunit [5].

The paper by Ray and coworkers in this issue describes the regulation of glutathione synthesis in response to oxidants in bronchial epithelial (NCI-H292) cells [6]. They found that exposure to menadione (MQ), a quinone which generates superoxide and H_2O_2 by redox cycling, decreases total cellular GSH content in bronchial epithelial cells. However, GSH content increased when the oxidative stress was withdrawn associated with persistent enhanced γ -GCS-HS mRNA expression. The menadione-induced induction of γ -GCS-HS mRNA was inhibited by actinomycin D, suggesting that γ -GCS-HS expression occurs at the transcriptional level. They concluded that NCI-H292 bronchial epithelial cells adapt rapidly and sensitively to oxidant stress, and this adaptive response is mediated by increased GSH synthesis. This may be an important

mechanism in bronchial epithelium allowing rapid adaptation to oxidative stress.

This study supports previous work which showed that exposure of bronchial (16-HBE) and alveolar epithelial (A549) cells *in vitro* to a range of oxidants, such as hydrogen peroxide, menadione, cigarette smoke condensate and hyperoxia provides an initial depletion of intracellular GSH, associated with increased formation of GSSG/GSH-conjugates, followed by a rebound increase in GSH at 24h [7–9]. These effects were also shown to be concomitant with increased expression of mRNA for the γ -GCS-HS gene in alveolar epithelial cells [7, 9, 10]. Thus, the short-term effects of various oxidants and oxidant-generating systems appear to up-regulate the gene for glutathione synthesis in alveolar epithelial cells, possibly providing a protective/adaptive mechanism against subsequent oxidative stress. However, the mechanism of oxidant-induced GSH synthesis was not studied in bronchial epithelial cells [11]. In this issue, Ray and coworkers found that exposure to menadione decreases total cellular GSH immediately followed by rapid induction of γ -GCS-HS mRNA expression at 3–6h in bronchial epithelial cells [6], whereas another study has shown that the induction of γ -GCS-HS mRNA occurs at 12h in alveolar epithelial cells [9]. It is possible that the GSH synthesis and tolerance mechanism that occur in response to oxidants may differ in various lung cells. Certainly the levels of GSH in the airspaces are different in different regions of the lungs being higher in more peripheral airspaces. It may also be possible that there is a wide variability in the cellular response to inhaled oxidants (e.g. hyperoxia and cigarette smoke) at different sites along the respiratory tract, which may be due to regional differences in the inherent metabolic potential of different lung epithelial cells to regulate intracellular GSH.

Exposure to sublethal doses of oxidants and oxidant-generating systems appear to initiate an adaptive intracellular anti-oxidant response [11, 12]. It would be important to determine whether this adaptive response is sufficient to protect cells against subsequent oxidative stress. This has been shown to occur following exposure to hyperoxia [13]. It is interesting that GSH levels in bronchoalveolar lavage are higher in asthmatics [14] suggesting a protective response to chronic oxidative stress.

The mechanism of adaptive response to oxidative stress in bronchial epithelial cells is not known. It has been reported that the expression of the γ -GCS-HS gene is regulated by the different regulatory signals in response to diverse stimuli including oxidants in specific cells. For example, the promoter (5'-flanking) region of human γ -GCS-HS gene is regulated by a putative c-Jun homodimeric complex-AP-1 sequence in alveolar epithelial cells (A549), a distal ARE containing an embedded phorbol myristate acetate (PMA)-responsive element (TRE/AP-1), and an electrophile responsive element (EpRE or its

functional equivalent, ARE) in liver cells (HepG2) [15–17]. These putative sites are induced by oxidative stress in various cell lines including human alveolar epithelial cells [15–17]. Therefore, it is likely that the expression of the γ -GCS-HS gene in bronchial epithelial cells is regulated by AP-1/ARE-mediated signal transduction mechanism in response to menadione. However this requires further investigation.

It is well known that γ -GCS activity can be modulated by the association of the catalytic heavy subunit with the regulatory light subunit. The γ -GCS-LS is also concomitantly induced in response to oxidative stress in rat lung epithelial L2 cells, suggesting that concomitant induction of both subunits may be a potential mechanism to enhance cellular GSH synthesis, and so develop cellular tolerance/adaptation to oxidative stress [18]. It remains to be determined whether γ -GCS-LS is also regulated by menadione in bronchial epithelial cells. Ray and coworkers show that the induction of γ -GCS-HS mRNA by sublethal oxidative stress was associated with increased γ -GCS activity and increased de novo GSH synthesis in a human bronchial NCI-H292 cell line [6]. This suggests that γ -GCS-LS is also induced by menadione in their system leading to increased γ -GCS activity.

The modulation of γ -glutamyl transpeptidase (γ -GT) may be another avenue for the regulation of intracellular GSH levels in lung cells. γ -GT cleaves extracellular GSH into its constituent amino acids and leads to the resynthesis of intracellular GSH rather than by direct intact cellular GSH uptake [19]. Thus, γ -GT acts as a salvage enzyme for cellular GSH synthesis. The lung epithelium has been shown to have high levels of γ -GT activity and utilizes extracellular GSH from the alveolar lining fluid [19]. As a result, γ -GT may be important in determining the levels of GSH in lung epithelial lining fluid (ELF). γ -GT expression is increased in rat lung epithelial L2 cells by oxidants such as menadione and tert-butyl hydroquinone [20], suggesting that γ -GT might play a role in the adaptive response against oxidative stress in bronchial epithelial cells. However, this contention needs further experiments.

The study of Ray and coworkers on the adaptive response of glutathione metabolism in response to oxidative stress should be interpreted cautiously because the study was carried out in NCI-H292 bronchial epithelial cells derived from a carcinoma rather than in primary bronchial epithelial cells. The responses to oxidant stress may differ somewhat from those of normal ciliated bronchial epithelial cells. Comparative studies using primary ciliated bronchial epithelial cells isolated from bronchial specimens of healthy individuals vs. patients with lung diseases would provide further insights into the inherent adaptive response imposed by oxidative stress. This may identify a susceptible population which may not respond for protective/adaptive regulation of glutathione synthesis. This population may be more prone to oxidative assault leading to lung inflammation/damage. Understanding of such molecular mechanisms of adaptive response of GSH synthesis in susceptible population will provide useful information for anti-oxidant augmentation therapeutic strategies.

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