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Changes in the glutathione system of lung cell lines after treatment with hydrocortisone

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Abstract Administration of anti-inflammatory glucocorticoids is a drug option in the therapy of acute respiratory distress syndrome (ARDS), according to present pathophysiological concepts. Surprisingly, glucocorticoids failed to show beneficial effects. This failure is not understood. In this investigation changes in the glutathione system due to hydrocortisone were found to consist of glutathione depletion and lowered glutathione reductase activities in alveolar epithelial type II cells, contrasted with unchanged activities in a fibroblast-like lung cell line. The glutathione system is thought to be the most important cellular antioxidative system and therefore alveolar epithelial type II cells might be more susceptible to oxidative stress after glucocorticoid treatment. As alveolar epithelial type II cells may be important targets in ARDS, because of their functions (stem cells of type I epithelial cells; surfactant synthesis), these changes might provide an explanation for the failure of glucocorticoids. In the present experiments the capability of hydrocortisone-treated alveolar epithelial type II cells to synthesise glutathione was found to be cysteine dependent at physiological concentrations. Transposing this observation to the in vivo situation, it might be expected that glucocorticoid efficacy in ARDS therapy requires co-administration of substances that increase glutathione synthesis, e.g. *N*-acetylcysteine.

Keywords Glucocorticoids · Lung fibroblasts · Alveolar epithelial type II cells · ARDS

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Introduction

Despite considerable effort in recent years, the acute respiratory distress syndrome (ARDS) still has a lethality of about 30–50% (Luhr et al. 1999). The syndrome is known to be a condition of host defence overreaction (Meduri and Kanangat 1999; Zimmerman et al. 1999). This involves immunological events leading to oxidative stress in lung tissue, and it is this inflammatory reaction that causes the crucial lung disorder.

As glucocorticoids are able to counteract inflammation at virtually all steps, a benefit for patients at ARDS should occur with such therapy. Surprisingly, no beneficial effects by various glucocorticoids (GCs) have been found in clinical trials (Bernard et al. 1987; Bone et al. 1989). Furthermore, antioxidative substances tested exclusively in ARDS were found to be effective in some animal studies only; they failed in clinical trials, however, as did GCs (Bernard et al. 1997; Domenighetti et al. 1999; Jepsen et al. 1992).

Inhalation of zinc-containing fumes may lead to a toxic lung oedema (Helm et al. 1971; Hjortsø et al. 1988), a subspecialty of the ARDS family. In cell culture experiments, zinc-mediated toxicity was increased in GC-treated alveolar epithelial type II cells and decreased in fibroblast-like lung cells (Walther et al. 1999a). Zinc-mediated toxicity is considered to be caused by inhibition of oxidised glutathione (GSSG) reductase and subsequent oxidative stress due to depletion of reduced glutathione (GSH) (Walther et al. 1999b). In accord with this, a decreased cellular glutathione content, e.g. after inhibition of synthesis, was accompanied by increased zinc-mediated toxicity (Walther et al. 1999b). In the present investigation effects of hydrocortisone (HC) on the glutathione system were examined. A decrease of cellular glutathione or a diminished disposal of GSH, due to altered GSSG reductase activity or de novo synthesis capacity in alveolar epithelial type II cells, might lead to a changed ratio of reduced/oxidised glutathione and could thereby explain the changed susceptibility to

zinc after HC treatment. Despite alveolar epithelial type II cells being rare in the lung, these cells are important in ARDS since they produce surfactant and are the stem cells of type I alveolar epithelial cells (Baritussio et al. 1992; Crapo et al. 1982). Both are needed in ARDS, especially when the alveolar region is reorganised. As glutathione is thought to be the most important cellular antioxidant (Reed 1990), such an explanation of decreased glutathione levels after HC treatment in alveolar epithelial cells would be important not only for zinc-mediated toxicity; changes in this system should influence other oxidative/antioxidative processes as well, which are thought to be important in ARDS, too.

In order to shed light on the ineffectiveness of GCs in ARDS we investigated effects of hydrocortisone on the GSH system in alveolar epithelial and fibroblast-like lung cells.

Materials and methods

Chemicals

Cell culture chemicals and other reagents were purchased as described previously (Walther et al. 1999a, 2000).

Trypan blue dye exclusion test

Cells were pretreated with HC for 72 h, then incubated with indicated concentrations of zinc chloride for 14 h. Afterwards, medium was discharged and cell layers were rinsed with 0.1% Trypan blue in phosphate-buffered saline (PBS) for 5 min. Trypan blue solution was removed and cells were counted and discriminated for colourless (vital) and blue cells.

Exposure protocol and cysteine incorporation assay

All cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA) and were grown as described previously (Walther et al. 1999a).

The investigations were performed in 24-well plates with 1.9 cm^2 of growth area per well. Cells were treated with 0–100 μmol/l hydrocortisone, as indicated, for 0–72 h. For the highest HC concentration an additional control containing 0.1% ethanol (corresponding to 100 μmol/l HC) was used. No differences were seen between controls and ethanol controls in any of the experimental studies.

After HC treatment, cells were incubated with radiolabelled cysteine (Cys) (^{35}S for 1 h, 1 μCi/cm^2 growth area, 15 or 50 μmol/l Cys) in minimum essential medium (MEM, Earle's salts). Cys incorporation was terminated by washing with ice-cold PBS and lysis of cell layers was induced with 150 μl 0.33 mol/l $HClO_4$. The acidic supernatant was used for thin layer chromatography (TLC) analysis and assay of glutathione content. The precipitate was dissolved in NaOH (0.5 mol/l, including 1% sodium laurylsulfate) and the acid-insoluble radioactivity was measured. Counting was performed in a TriCarb 2500 liquid scintillation counter (Canberra Packard, Frankfurt/Main, Germany) using Ultima Gold XR (Canberra Packard) as the liquid scintillation cocktail.

Measurement of glutathione and GSSG reductase activity

After treatment of cells with HC, total glutathione and oxidised glutathione were measured by the glutathione reductase recycling assay, as described by Tietze (1969), using Elman's reagent [5,5'-dithio-*bis*(2-nitrobenzoic acid)] and nicotinamide-adenine dinucleotide phosphate reduced form (NADPH), with slight modifications (Walther et al. 2000).

Reductase activity was measured using a modified assay for glutathione quantification as described previously (Walther et al. 2000).

TLC analysis of GSH metabolites by TLC separation

After Cys incorporation, 4 μl of the acidic extract were transferred to 10×10 cm TLC sheets (Silicagel 60 F; Merck, Darmstadt, Germany) and developed in phenol:1-butanol:acetic acid (9:3:2). TLC Rf values of commercially available GSSG, cystine, γ-glutamylcysteine, GSH, and Cys were estimated to be 0.04, 0.07, 0.125, 0.13, and 0.26, respectively. After chromatography, TLC sheets were dried and placed on BAS-MP imaging plates (Fuji, Düsseldorf, Germany) and exposed for 2–7 days. Afterwards, imaging plates were scanned by the Fujix BAS 1000 Bioimaging Analyzer system (Fuji) with the TINA software package (v. 2.07). Radioactivities of regions of interest were estimated after processing a vertical section of each radioactivity track.

Determination of cellular volumes

Cell layer volumes were estimated by determination the volume of distribution of ^3H-labelled water. Cell layers were incubated with MEM containing ^3H_2O for 1 h at 36°C, then washed three times with ice-cooled PBS (about 10 min). Afterwards, cell layers were lysed with 0.5 *N* NaOH and radioactivity was measured. Volumes were calculated according to initial radioactivity of MEM. Alternatively, cell layer volumes of non-HC-treated cells were measured by counting of cells and calculating the single cell volume after assessment of the mean cell diameter.

Protein determination

Protein content of cell layers were measured according to a modified Bradford procedure (Read and Northcote 1981), as described previously (Walther et al. 1999a).

Statistical evaluation

Mean and standard deviation (SD) of at least three experiments were calculated and statistical differences were estimated by analysis of variance with the method of last significant difference (ANOVA-LSD) or by *t*-test for paired variables. Additionally, correlation between zinc toxicity and HC efficacy was calculated by Spearman's rank-order test.

Results

Effect of hydrocortisone on zinc-mediated loss of viability

Viability of L2, A549 and 11Lu cells was measured by the Trypan blue dye exclusion test. The value for non-HC-treated controls (98 ± 3%) was not affected by HC (data not shown). After cells had been exposed to zinc chloride for 14 h, viability decreased in a concentration-dependent manner. When cells had been pretreated with hydrocortisone for 72 h, the decreased viability due to zinc exposure was significantly amplified in alveolar

epithelial L2 cells at 50 and 60 μmol/l zinc chloride, and in A549 cells at 75 μmol/l zinc. By contrast, loss of viability in the fibroblast-like 11Lu cells due to 50 or 60 μmol/l zinc exposure was significantly decreased after hydrocortisone pretreatment (Table 1).

Effect of hydrocortisone on cellular total glutathione content

Control cell layers of A549, L2 and 11Lu cells contained 92 ± 35, 20 ± 8 and 29 ± 7 pmol glutathione/μg protein, respectively. Similar glutathione contents were found after incubation of cells with up to 100 μmol/l HC for about 15 min. After cell layers had been treated with HC for 24 h, a dose-dependant decrease of cellular glutathione content occurred in all three cell lines (Fig. 1a). The decrease was significant at the level of 0.1 μmol/l HC. After the 48-h treatment period, no different glutathione content was found in the fibroblast-like cell line compared to the controls, while a decrease was even more pronounced in alveolar epithelial cells relative to the 24 h period (data not shown). When cell layers were treated with HC for 72 h the decrease of glutathione in A549 and L2 cells was further enhanced compared to the 48 h period. Again, no differences in fibroblast-like 11Lu cells were found relative to controls (Fig. 1b).

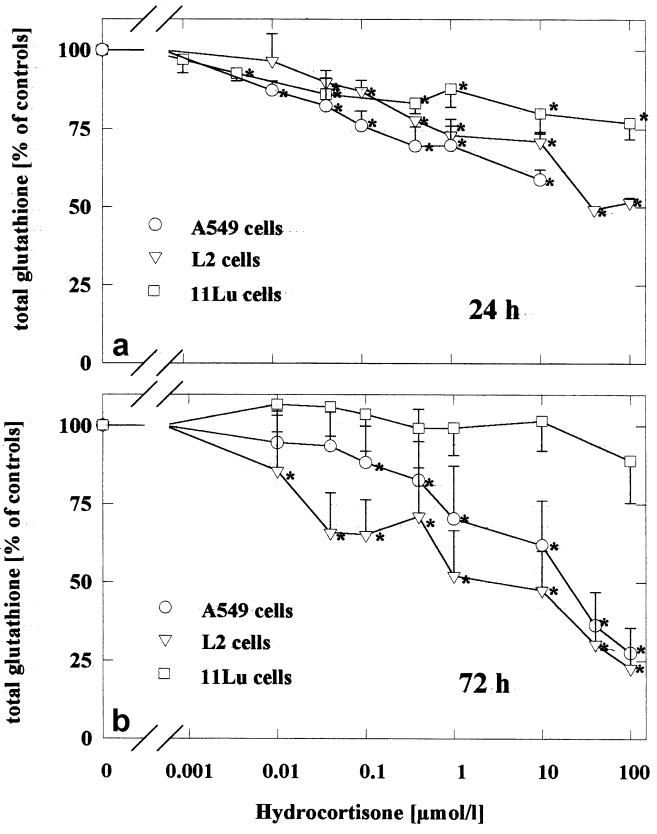

Fig. 1a, b Cellular glutathione content of lung cell lines after treatment with hydrocortisone. Cells were treated with up to 100 μmol/l hydrocortisone in normal growth medium for 24 h (**a**) or 72 h (**b**). Glutathione was measured in perchloric acid extracts and expressed as percentage of controls. Means ± SD of 6–10 independent experiments are given. *$p < 0.05$, ANOVA-LSD

Effect of hydrocortisone on cellular GSSG content

About 3% and 1% of total glutathione was determined as GSSG for non-malignant and malignant cell lines, respectively. This fraction of GSSG was not changed in non-malignant fibroblast-like 11Lu cells and malignant A549 cells after HC treatment. In L2 cells GSSG was raised slightly from 3.1 ± 2.0% (controls) to 4.4 ± 5.4% in cells treated with 100 μmol/l HC due to decreased total glutathione, but the cellular amount of GSSG was not increased.

Table 1 Trypan blue dye exclusion measure of cell viability following zinc exposure after pretreatment with hydrocortisone for 72 h. Alveolar epithelial (A549, L2) and fibroblast-like (11Lu) lung cells were pretreated with the indicated concentrations of hydrocortisone for 72 h in normal growth medium. Afterwards, cells were incubated with zinc chloride as indicated for 14 h in minimum essential medium, and viability was assessed by the Trypan blue exclusion test. Means ± SD of three or four independent experiments are shown

Cell line	$ZnCl_2$ (μmol/l)	Concentration of hydrocortisone (μmol/l)					Significant tendency
		0	0.1	1	10	100	
A549	0	99 ± 0	99 ± 1	100 ± 1	100 ± 0	100 ± 0	NS
	40	97 ± 4	–	95 ± 10	99 ± 1	99 ± 1	NS
	75	87 ± 15	85 ± 23	78 ± 15	71 ± 17	50 ± 25	*
	100	63 ± 23	73 ± 24	60 ± 23	54 ± 13	51 ± 28	NS
L2	0	100 ± 1	100 ± 1	–	100 ± 1	99 ± 1	NS
	50	98 ± 2	81 ± 16	–	76 ± 22	69 ± 33	*
	60	85 ± 22	54 ± 44	–	42 ± 33	31 ± 30	*
	70	40 ± 34	15 ± 33	–	9 ± 14	3 ± 5	NS
11Lu	0	99 ± 1	99 ± 1	97 ± 3	–	98 ± 1	NS
	40	40 ± 28	50 ± 37	53 ± 41	–	71 ± 28	NS
	50	11 ± 13	22 ± 28	37 ± 34	–	69 ± 35	*
	60	2 ± 2	23 ± 36	47 ± 32	–	38 ± 36	*

*$p < 0.05$, Spearman's rank-order correlation (*NS* not significant)

Effect of hydrocortisone on cellular GSSG reductase activity

Activity of GSSG reductase (GR) of control cell layers was 6000 μU/cm^2 (A549), 500 μU/cm^2 (L2), or 1500 μU/cm^2 (11Lu). No changes of GR activity were found after incubation with HC for 24 h (Fig. 2a). Activity had begun to decrease in A549 and L2 cells after a treatment period of 48 h (data not shown). After cells had been treated with HC for 72 h, a significant decrease was found at HC concentrations of 0.4 μmol/l (A549) or 0.1 μmol/l (L2) (Fig. 2b). In fibroblast-like 11 Lu cells, a significant increase in GR activity was found at concentrations above 0.01 μmol/l HC. When GR activity is expressed per protein content, no changes in activities were found for any cell line (Fig. 3).

Effect of hydrocortisone on glutathione synthesis

The cell lines tested synthesised 32 ± 20 (11Lu), 50 ± 26 (A549), or 23 ± 13 nmol GSH/mg protein (L2) in 1 h when 50 μmol/l cysteine was added. When 15 μmol/l cysteine was added during the cysteine incorporation study, synthesis rates dropped to about 10–20% of that at the 50 μmol/l concentration. After cells had been treated with HC no change, and particularly no decrease, in synthesis rates was found (Fig. 4).

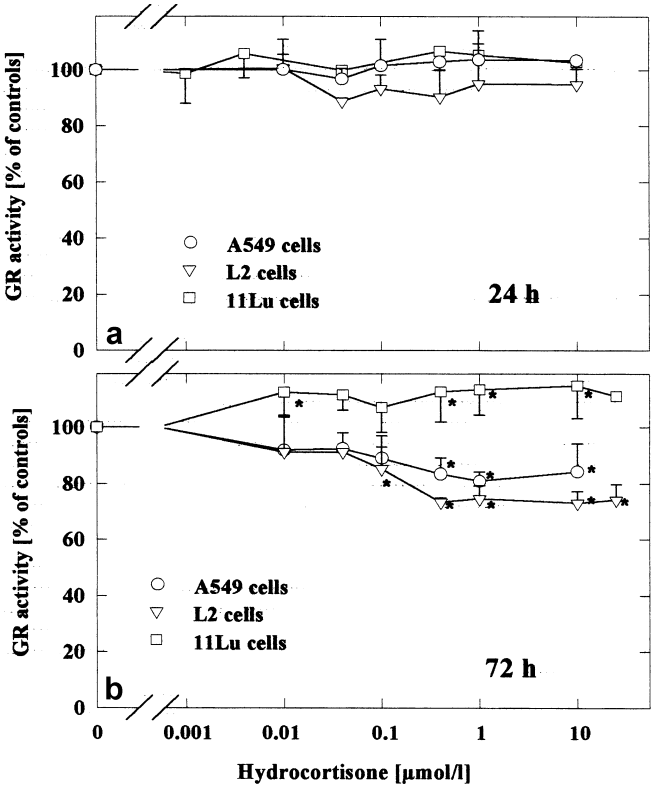

Fig. 2a, b Oxidised glutathione reductase (GR) activity of lung cell lines after treatment with hydrocortisone. Cells were treated with up to 100 μmol/l hydrocortisone in normal growth medium for 24 h (**a**) or 72 h (**b**). GR activity was measured in Triton X-100 cell lysates and expressed as percentage of controls. Means ± SD of three or four independent experiments are given. *$p < 0.05$, ANOVA-LSD

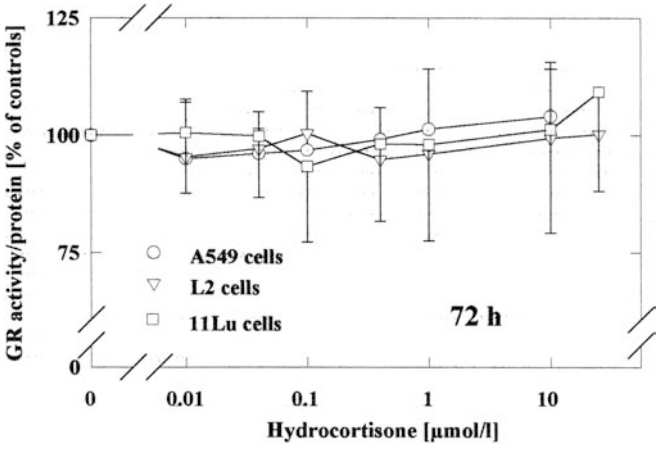

Fig. 3 Oxidised glutathione reductase (GR) activity per unit of protein in lung cell lines after treatment with hydrocortisone. Cells were treated with up to 100 μmol/l hydrocortisone in normal growth medium for 72 h. GR activity was measured in Triton X-100 cell lysates as related to protein content and was expressed as percentage of controls. Means ± SD of three or four independent experiments are given

Influence of GSSG reductase addition on activity in cell lysates

Cells were lysed with Triton X-100 and diluted to obtain activity of about 2 mU per sample (in each experiment the same amount). In A549, L2, and 11Lu cells, activities of 1.4 ± 0.2, 2.2 ± 1.1, and 3.6 ± 1.3 mU per sample were used, respectively. Then, reductase activity (bovine, mucosa) between 1 and 4 mU was added and total activity was measured. No differences were seen between expected and measured activities in L2 and 11Lu cells (Fig. 5b,c), whereas in A549 cells the activity measured was significantly lower than expected (Fig. 5a). In HC-treated A549 cells, too, total activity found was lower than expected, but differences from the expected values were not significant.

Influence of hydrocortisone on cell volume

Estimated volumes of 1.9-cm^2 cell layers of A549, L2 or 11Lu cells were 370 ± 125, 210 ± 60, or 215 ± 60 nl, respectively, when measured as volume of water distribution (Table 2). From an estimate of volumes using cell numbers and single cell volume, an equivalent volume for each control cell layer was calculated (Table 2). Volumes were significantly increased in L2 cells after treatment with 0.01 to 1 μmol/l HC for 24 or 48 h. In addition, volumes were increased in 11Lu cells with concentrations of HC up to 1 μmol/l. No changes in A549 cell layer volumes were found (Fig. 6).

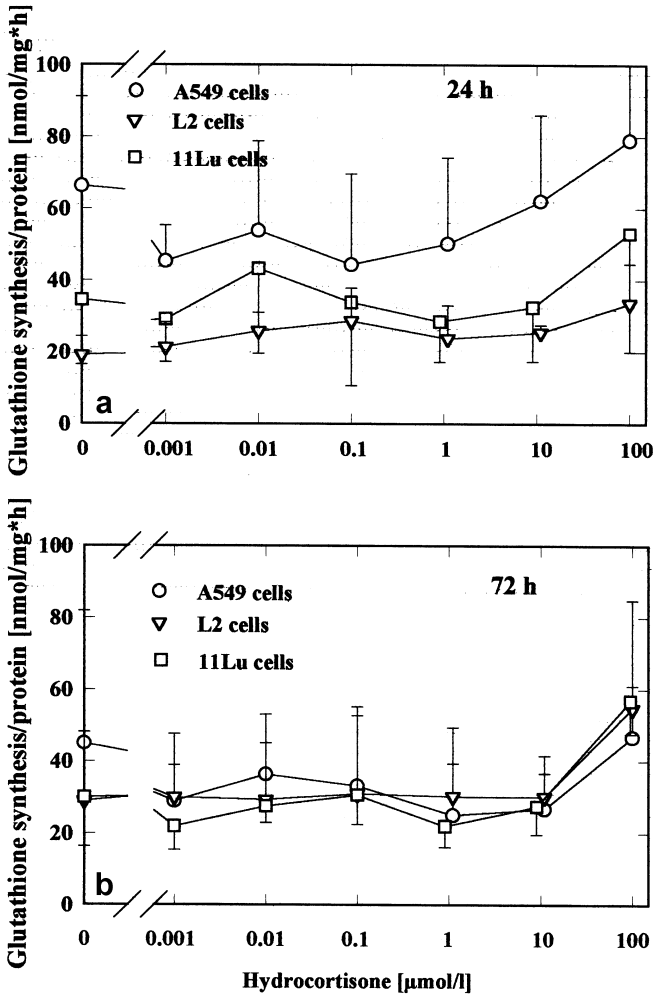

Fig. 4a, b Influence of hydrocortisone treatment on glutathione (GSH) synthesis rates in lung cell lines. Cell layers were treated with hydrocortisone for 24 h (**a**) or 72 h (**b**). GSH synthesis rates (per hour) were measured after incorporation of 50 µmol/l radioactive cysteine into cellular glutathione fraction. Means ±SD of four independent experiments are given

Discussion

Glucocorticoids are known to reduce zinc resistance of alveolar epithelial type II-like cells and to increase resistance in lung fibroblast-like cells (Walther et al. 1999a). This effect has previously been demonstrated using methionine incorporation inhibition, or lactate dehydrogenase leakage, and is also shown by Trypan blue dye exclusion test in the present investigation (Table 1). Zinc-mediated toxicity was linked to the GSH system (Mize and Langdon 1962; Steinebach and Wolterbeek 1993). Therefore this investigation tested whether different changes in zinc susceptibility observed after HC treatment could be caused by changes in the GSH system. GCs are known to increase GSH levels in some cells, e.g. hepatocytes, HeLa cells, fibroblasts, or renal tubular cells (Cai et al. 1995, 1997; Chobert et al. 1996; Lu et al. 1992; Millar and Jinks 1985; Wolff et al. 1996). Therefore, it was surprising to find a decrease in GSH content of the alveolar epithelial cells in this study. Only the ocular lens is known to show a decrease in GSH levels after GC treatment, which might lead to cataracts as observed in man and animal (Lee et al. 1998; Setogawa et al. 1994). On the other hand, Rahman et al. (1999) also found a decrease of glutamylcysteine synthetase heavy chain (the catalytic subunit of the rate limiting enzyme in glutathione synthesis) in A549 cells, leading to a decreased GSH content, after dexamethasone administration. Altogether GCs might commonly increase GSH in organs, while in the lens and in alveolar epithelial type II-like cells a decrease of GSH content occurs due to GC treatment.

The question arises, what mechanism is responsible for the decreased GSH levels? No increase in cellular GSSG was found in HC-treated cells, while the GSSG/GSH ratio increased due to decreased GSH content. Furthermore a decrease in the glutathione content of the medium was found in this study in all three cell lines (data not shown), corresponding to the decreased cellular content of glutathione in alveolar epithelial cells after HC treatment. Therefore an increase in oxidation of GSH after HC treatment might be excluded as a mechanism.

Capacity to provide GSH from de novo synthesis is about one-third of the total cellular GSH disposal. Additionally, the effect of decreased cellular content develops within 20–60 h. Therefore a suitable explanation might be the decreased rate of synthesis, corresponding to the findings of Rahman et al. (1999) as cited above.

Increased cysteine availability is commonly used to increase GSH content. This is very suitable because the K_m of glutamylcysteine synthetase (the rate limiting enzyme in GSH synthesis) is in the order of about 200 µmol/l for Cys, while cellular concentrations are lower but increase with increasing plasma concentrations (Ammon et al. 1992; Huang et al. 1993) (normal levels 10 µmol/l). Consequently, oral *N*-acetylcysteine, commonly given as a cysteine precursor, increases GSH concentrations in many organs and tissues, especially if concentrations had been lowered before (Burgunder et al. 1989). In our investigation, GSH synthesis rates were measured at cysteine concentrations of 15 and 50 µmol/l. Synthesis rates at 50 µmol/l Cys were about 2- to 3-fold those at 15 µmol/l, but were not changed by HC. Only low molecular weight glutathione metabolites are detectable with the extraction and detection method used, while protein or glutathione–protein conjugates were not noticeable. As such conjugates are produced especially under conditions of oxidative stress, their content should be increased (if at all changed) in HC-pretreated type II alveolar epithelial cells as GSSG/GSH ratio rises. Therefore, in HC-treated type II cells, GSH synthesis might be even more underestimated and no evidence for GSH synthesis restriction would be hidden.

It might be concluded that the decreased GSH content caused by HC can be counteracted through

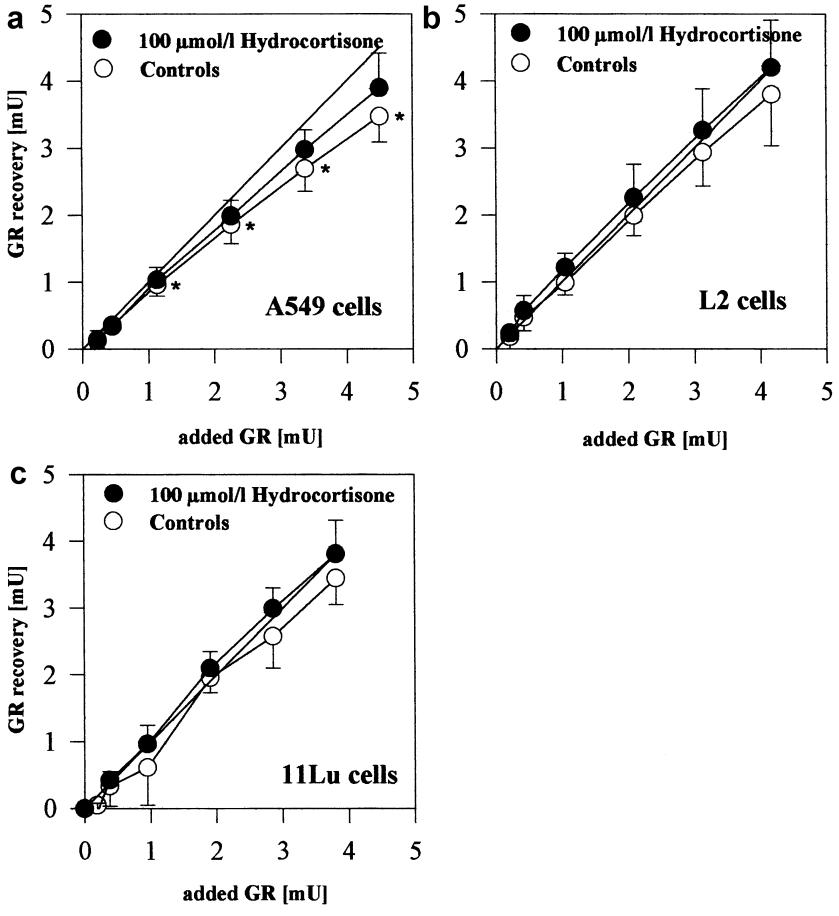

Fig. 5a–c Oxidised glutathione reductase (GR) recovery in lysates of lung cell lines A549 (**a**), L2 (**b**) and 11Lu (**c**). Cells were treated with 100 µmol/l hydrocortisone for 72 h. Controls and hydrocortisone-treated cell layers were lysed with Triton X-100 to yield GR activities of about 2 mU per fraction. Bovine GR was added and total activity was measured. Recovery of added activity from this fraction is shown. Means ± SD of four (A549) or three (L2, 11Lu) independent experiments are given. $*p < 0.05$, *t*-test for paired variables

increased synthesis rates by increasing cysteine availability. In accordance with this we found significantly decreased zinc-induced toxicity in L2 cells (controls and HC-pretreated) as a result of *N*-acetylcysteine administration (manuscript in preparation).

In the present investigation, a change in GR activity was found for the HC-treated alveolar epithelial cells in addition to decreased GSH contents. Therefore, the question arises whether the specific enzyme activity or the enzyme amount has changed. As different isoenzymes of GR are not known (Goldberg and Spooner 1987) this question might be focussed on existing effectors regulated by GC. In non-malignant alveolar epithelial L2 and fibroblast-like 11Lu cells, the expected sum of detected GR activities of cell lysates and exogenously added activity was found (Fig. 5b,c). Therefore, no evidence was found for a surplus or deficiency of effectors of the GR enzyme. In malignant A549 cells, the total GR activity of a known cellular activity plus exogenously added activity did not reach the expected values (Fig. 5a). This effect was less marked in HC-treated cells than in controls. In all cell lines nearly the same cellular activity was used and, therefore, any non-linear, decreased activity measured at high GR activities as a result of the detection method can be excluded. Therefore an inhibitor of GR activity in malignant A549 cells might exist, which is specifically diluted if more enzyme is present. On the other hand, as A549 cells contain much more GR than the non-malignant cells tested, a marked decrease in protein content was present in assays of A549 cell lysates compared with that of the non-malignant cells. Altogether an unspecified activation of GR due to protein or the presence of an unspecified inhibitor, which itself is inhibited by proteins, might be a possible explanation. When GR activity is expressed relative to cellular protein content, no changes are found as a result of HC treatment of cells (Fig. 3). Therefore, the best explanation of decreased GR activity of HC-treated alveolar epithelial cells might be given as being due to the catabolic action of GCs. This therefore is expected to occur in vivo too, whereas

Table 2 Volumes of 1.9-cm^2 cell layers of alveolar epithelial (A549, L2) and fibroblast-like (11Lu) lung cells. Cell layer volumes of 1.9-cm^2 growth area were measured as the water distribution volume of ^{3}H-water or as the product of cell numbers and single cell volume as assessed after determination of the mean cellular diameter of at least 100 cells of each cell layer. Means ± SD of 3–6 independent experiments are given as nanolitres of each cell layer

Cell line	Cell numbers × single cell volume	Water distribution volume
A549	520 ± 57	371 ± 127
L2	189 ± 61	208 ± 57
11Lu	230 ± 60	216 ± 62

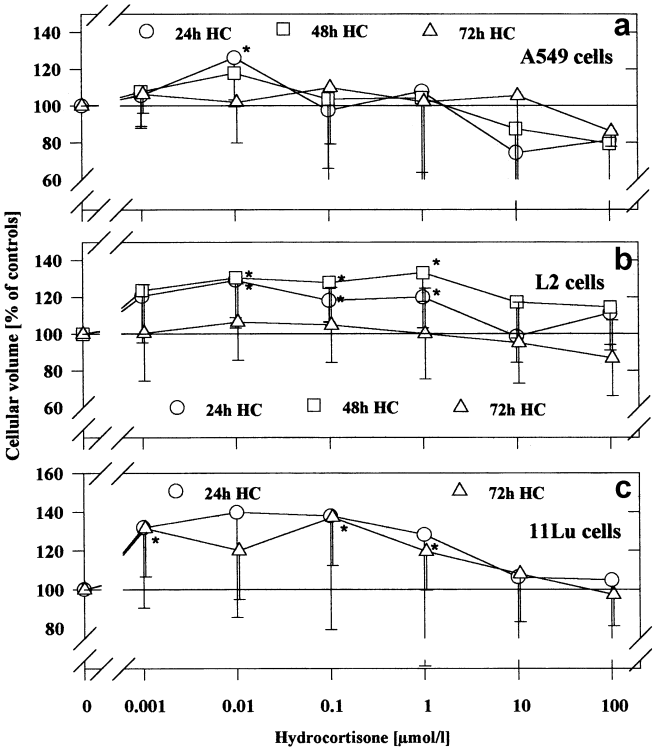

Fig. 6a–c Influence of hydrocortisone on cell layer volumes of lung cell lines A549 (**a**), L2 (**b**) and 11Lu (**c**). Cells were treated with hydrocortisone for up to 72 h. Volume of water distribution was measured by addition of ^{3}H-labelled water and expressed as percentage of control. $*p < 0.05$, ANOVA-LSD

changed activity by protein-associated effectors might be expected to be in vitro (cell culture) artefacts.

Most reactions of GSH might depend on the GSH/GSSG ratio representing the cellular redox potential. Some effects might really depend on GSH concentrations. Therefore, differences due to HC treatment in GSH amount and volume of cell layers were detected. All observed effects depending on GSH concentrations were decreased because of the increased cellular volume, in addition to the decreased GSH content due to HC. These volume effects might decrease GSH concentrations by a further 20–30% in addition to decreased GSH amounts as assessed for 0.01–1 µmol/l HC in 11Lu and L2 cells. A similar effect of dilution can be marked for GR. The decrease in availability of this cytosolic enzyme, therefore, should be meaningful especially in situations of oxidative stress, when amounts of GSSG increase.

Measurement of cell layer volume by water distribution might be critical because of the washing procedures, resulting in biased values if temperature (and time) of washing is not correctly heeded. Thus, values were only expressed as percentage of controls and all samples of each experiment were treated equally. Nevertheless, no significant differences of values assessed by water distribution volume and those assessed by cell amount and single cell volume (assessed by microscopically measured diameter) were found (Table 2).

The decrease in GSH content and GR activity should be kept in mind not only for zinc-exposed lungs. The GSH system is known to be the most important antioxidative cellular system. This is important for ARDS, too, as it is accompanied by oxidative stress in pulmonary cells. Alveolar epithelial type II cells are important target cells in ARDS because they are the stem cells for type I epithelial cells and they synthesise surfactant. Therefore, the HC-mediated GSH decrease might be important in ARDS therapy (with glucocorticoids) as well. Concerning the HC concentrations tested, it was evident that the highest HC concentration of 100 µmol/l was associated with significantly changed GR activities, GSH contents and zinc susceptibility in vitro. Such a concentration of the GC (or ones even higher) can be estimated for lung tissue after recommended systemic and inhalation application (Helm et al. 1971).

Altogether, these investigations on GSH content seem to be worthwhile since GSH is known to be decreased in alveolar epithelial lining fluid in ARDS (Bunnell and Pacht 1993). Furthermore, an increase of cellular GSH seems to be effective especially in some ARDS models based on animal studies (Bernard et al. 1997; Laurent et al. 1996; Moss et al. 2000; Ortolani et al. 2000; Suter et al. 1994; Walmrath et al. 2000). Our investigations point to an increased susceptibility of HC-treated alveolar epithelial cells towards oxidative stress caused by a decreased GSH content (as well as a decreased GR activity due to the catabolic action of the GC). The results presented in this investigation, therefore, might explain the failure of glucocorticoids in the therapy of ARDS, at least in part due to a selective increased damage of alveolar epithelial cells as a result of oxidative stress. As type II alveolar epithelial cells are even rare cells of the lung, such a postulated increased damage of this cell type in HC-treated ARDS might not be detectable by commonly used biochemical assays. Further examinations should test combinations of GCs and glutathione precursors, such as *N*-acetylcysteine, as a therapeutic option in special ARDS models.

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