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Adaptation to acrolein through upregulating the protection by glutathione in human bronchial epithelial cells: The materialization of the hormesis concept

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ABSTRACT

Acrolein is a thiol reactive compound present in cigarette smoke and plays a pivotal role in the deleterious effects of smoking. Acrolein causes toxicity in human bronchial epithelial cells in a dose dependent manner. GSH forms the first line of defense against acrolein-induced toxicity. At high doses of acrolein ($\geqslant$10 μM) the capacity of the cellular protection by GSH is overwhelmed and GSH is not able to quench all the acrolein, resulting in cytotoxicity.

At a relatively low dose of acrolein (3 μM), no cytotoxicity is observed due to protection by GSH. Moreover we found that exposure to a low dose of acrolein protects cells against the toxic effect of a second higher dose of acrolein. The adaptation to acrolein is induced via Nrf2 mediated gene expression of γ-glutamylcysteine synthetase leading to elevated GSH levels. This upregulation of the protection by GSH demonstrates a hormetic response to acrolein.

Hormesis is an adaptive or compensatory response induced by a relatively subtle challenge of homeostasis by a toxic compound. Insight into the mechanism of hormesis is mandatory for a more accurate societal regulation of toxic compounds.

1. Introduction

The human body is continuously exposed to a wide variety of compounds present in the environment that can have toxic effects at a relatively low dose, such as polycyclic aromatic hydrocarbons, aldehydes and metals [1]. These exogenous compounds can be inhaled by breathing polluted air, ingested by consuming food or contaminated water or absorbed via the skin. A relatively high dose of an exogenous compound results in damage to vital macromolecules including critical proteins and DNA, causing toxicity. This toxicity has been implicated in the etiology of cardiovascular, respiratory and neurodegenerative diseases [2]. In the attempt to prevent disease formation, governmental agencies regulate upper limits at which humans can safely be exposed.

The continuous exposure to toxicants seems to render us as relatively resistant to these substances, which evidences our ability to adapt. The extent of the ability to adapt defines the status of human health [3]. The phenomenon of inducing an adaptive or compensatory response by a relatively mild challenge on homeostasis is called hormesis [4]. To actually examine the concept of hormesis, we have selected acrolein as an exogenous compound. Acrolein, an α,β-unsaturated aldehyde, is implicated in the toxicity of cigarette smoke, traffic exhaust and other air pollution [5]. Acrolein can also directly induce cellular damage [6] due to its intrinsic reactivity [7,8]. According to the Hard Soft Acid Base concept acrolein is a soft electrophile that prefers to react to soft nucleophiles such as thiols [9]. The high thiol reactivity of acrolein results in Michael adducts of acrolein on cysteine residues of critical cellular proteins which can elicit cell toxicity [7]. Acrolein can be considered as a toxicophore, a characteristic moiety within molecules responsible for their toxic properties, and is therefore a model for electrophilic xenobiotics that display toxicity as a direct result of their electrophilicity, e.g. NAPQI [10]. In this study the concept of hormesis is investigated on a cellular level by assessing the adaptive response to acrolein in bronchial epithelial cells.

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2. Materials and methods

2.1. Chemical reaction acrolein and GSH

The amount of acrolein (Sigma–Aldrich, St. Louis, MO, USA) reacting with reduced glutathione (GSH) (Sigma–Aldrich, St. Louis, MO, USA) was determined by adding 0, 1, 3, 6, 9, 10 and 12 μM acrolein to 10 μM GSH at 37 °C. The amount of acrolein that has reacted with GSH is indirectly measured by quantifying the amount of GSH that has not reacted with acrolein. A concentration of 120 μM of DTNB (Sigma–Aldrich, St. Louis, MO, USA) is added and the absorbance is spectrophotometrically determined at a wavelength of 412 nm.

2.2. Cell culture

Adenovirus-12 SV40 hybrid virus transformed, non-tumorigenic human bronchial epithelial cells (BEAS-2B; ATCC, Manassas, USA) were cultured in Dulbecco's Modified Eagle's Medium (DMEM/F-12; Gibco, Bleiswijk, The Netherlands) supplemented with 50 U/ml penicillin (Gibco, Bleiswijk, The Netherlands), 50 μg/ml streptomycin (Gibco, Bleiswijk, The Netherlands), 15 μg/ml bovine pituitary extract, 0.5 mg/ml bovine serum albumin (Invitrogen, Breda, The Netherlands), 10 ng/ml cholera-toxin (List Biological Laboratories, Inc., Campbell, California), 10 ng/ml epidermal growth factor (Merck Millipore, Darmstadt, Germany), 5 μg/ml insulin, 5 μg/ml transferrin and 0.1 μM dexamethasone (Sigma–Aldrich, St. Louis, MO, USA) in an environment containing 95% O_2 and 5% CO_2 at 37 °C. The maximal passage used in this experiment was 10.

2.3. Treatments

Cells were seeded in 6-well plates at a density of 400,000 cells per well and cultured overnight in 95% O_2 and 5% CO_2 at 37 °C. Treatments with acrolein were made in Hank's Balanced Salt Solution (HBSS; Gibco, Bleiswijk, The Netherlands). Cells were exposed to 0, 1, 3, 10, 30, 100 and 200 μM acrolein for 30 min and lysed immediately to determine GSH levels. To determine cellular toxicity HBSS was replaced with medium and 24 h post acrolein exposure medium was collected to measure lactate dehydrogenase (LDH) activity of the released LDH. In addition, cells were treated with 3 μM and 10 μM of acrolein and GSH levels were determined 4 and 6 h after exposure (Supplementary Fig. 1). Furthermore, 4 and 8 h after exposure RNA was isolated to determine gamma-glutamylcysteine synthetase (γGCS) expression (Supplementary Fig. 1). Finally, 3 μM of acrolein was added to the cells 4 h prior to 10 μM of acrolein treatment and GSH levels and cellular toxicity were measured (Supplementary Fig. 1).

2.4. Detection of cytotoxicity

LDH activity was measured by transferring 50 μl of the collected medium to a 96-well plate. A solution of 100 mM sodium pyruvate in 500 mM potassium phosphate buffer, pH 7.5 was prepared. After addition of 50 μl of 0.25 mg/ml NADH in sodium pyruvate solution, the change in absorbance at a wavelength of 340 nm was determined over 4 min using a Spectramax plate reader (SpectraMax M2 & M2e Multi-Mode Microplate Reader, Sunnyvale, United States). LDH activity of the samples was compared to the LDH activity measured after LDH leakage due to cell lysis achieved by addition of 3% Triton-X-100 (Sigma–Aldrich, St. Louis, MO, USA) for 15 min.

2.5. Analysis of cellular GSH levels

Cells were washed with 2 ml 1× Dulbecco's Phosphate Buffered Saline (DPBS; Gibco, Bleiswijk, The Netherlands). The cells were lysed with 500 μl 100 mM potassium phosphate buffer containing 10 mM EDTA disodium salt, pH 7.5 and 1% Triton-X-100 (Sigma–Aldrich, St. Louis, MO, USA). After 30 min of incubation on ice, cells were scraped and then centrifuged for 10 min at 14,000 rpm 4 °C to remove cellular debris. The protein content was measured using the bicinchoninic acid assay (BCA; Pierce, Thermo Fisher Scientific, Etten-Leur, The Netherlands). In addition, 300 μl of remaining supernatant was mixed 1:1 with 6% sulfosalicylic acid (Sigma–Aldrich, St. Louis, MO, USA) and samples were diluted 1:5 in 100 mM potassium phosphate buffer with 10 mM EDTA disodium salt, pH 7.5. Finally, GSH was determined using an enzymatic recycling method as previously described [11].

2.6. Measurement of gamma-glutamylcysteine synthetase expression

First cells were lysed using Qiazol (Qiagen, Venlo, The Netherlands). Second, 200 μl chloroform (Sigma–Aldrich, St. Louis, MO, USA) per ml of Qiazol was added to perform a phase separation. After incubation for 2–3 min at 20 °C and centrifugation for 15 min at 12,000*g* 4 °C, the colorless aqueous upper phase containing RNA was removed and added to 500 μl isopropanol per ml of Qiazol to allow the RNA to precipitate. The cells incubated at 4 °C overnight and after centrifugation the supernatant was removed. The pellet was washed with 1 ml 75% ethanol per ml of Qiazol. After centrifugation at 7500*g* for 5 min at 4 °C, the supernatant was removed and the pellet was allowed to dry for an hour. Hereafter, the pellet was resuspended in 50 μl of RNAse/DNAse free water and incubated for 10 min at 60 °C. The quantity of RNA was determined using the nanodrop (thermo scientific nanodrop 1000 spectrophotometer, isogen life science, De Meern, The Netherlands). Five hundred nanograms of RNA was converted into complementary DNA (cDNA) by using iScript cDNA synthesis kit (Biorad, Veenendaal, The Netherlands). Sensimix SYBR & Fluorescein kit (Bioline, Alphen aan de Rijn, The Netherlands) was used to perform quantitative RT-PCR measuring gamma-glutamylcysteine synthetase (γGCS; sense: 5′-GCACATCTACCACGCCGTC-3′ and antisense: 5′-CCACCTCATCGCCCCAC-3'). β-actin (β-actin; sense: 5′-CCTGGCACCCAGCACAAT-3′ and antisense: 5′-GCCGATCC ACACGGAGTACT-3′) was applied as housekeeping gene. Finally, the $2^{-\Delta\Delta CT}$ method was used to determine relative γGCS gene expression [12].

2.7. Statistics

All data are shown as mean ± SEM. A *t* test was performed for independent samples with equal variances to assess statistical significance between two individual groups. A $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. The reactivity of acrolein towards GSH

To investigate the reaction of acrolein towards GSH, various amounts of acrolein were added to a fixed amount of GSH (initial concentration of 10 μM in a volume of 1 ml) in a test tube. This revealed that acrolein reacts within seconds with GSH in stoichiometry of 1:1 (Fig. 1).

3.2. Acrolein depletes GSH in the cell

To investigate whether acrolein is quenched by intracellular GSH similarly as in the test tube, BEAS-2B cells were treated with acrolein (1, 3, 10 and 30 μM), and GSH was measured. Acrolein dose-dependently reduced intracellular GSH in BEAS-2B cells

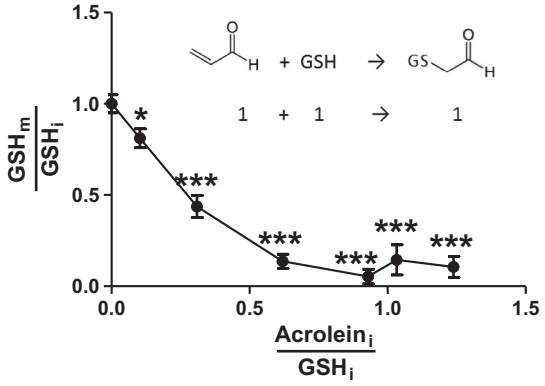

Fig. 1. The chemical reaction of acrolein and GSH. Variable amounts of acrolein were added to a fixed amount of GSH (GSH_i; the initial amount of GSH is 10 nmol GSH that corresponds to 10 μM GSH in 1 ml). Both the initial amount of acrolein ($Acrolein_i$) and the amount of GSH measured (GSH_m) are shown relative to the initial added fixed amount of GSH (GSH_i). Experiments are at least performed in triplicate and data are shown as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to control (=0 μM of acrolein).

(Fig. 2A). The stoichiometry, i.e. reduction in the amount of GSH in the cell (nmol) relative to the amount of acrolein (nmol) added was lower compared to that in the test tube. With addition of 1 μM acrolein the ratio between the amount of GSH consumed and the amount of acrolein added appeared to be 0.74:1 (Fig. 2A). At higher concentrations of acrolein, this ratio further dropped (Fig. 2A). For example, with 10 μM of acrolein the ratio of GSH consumption versus acrolein added was 0.18:1. When compared to a 1:1 stoichiometry (acrolein:GSH), the relative amount of acrolein that was not captured by intracellular GSH was calculated. This calculation illustrated that as acrolein concentrations increased, the relative amount of acrolein that was not quenched by intracellular GSH also increased. When 10 μM of acrolein was added to BEAS-2B cells approximately 24 nmol of the originally added 30 nmol of acrolein did not react with GSH (Fig. 2B), which corresponds to 80% of the acrolein added.

3.3. Acrolein induces cytotoxicity

To identify at what threshold acrolein becomes cytotoxic, BEAS-2B cells were exposed to a range of acrolein concentrations and LDH was measured. Addition of ⩽3 μM of acrolein did not significantly induce cytotoxicity. Upon raising the concentration of acrolein above 3 μM, acrolein dose-dependently induced cytotoxicity, evidenced by LDH leakage (Fig. 2C). Relating cytotoxicity to the amount of acrolein showed that acrolein had a threshold between 22 and 75 fmol/cell (3–10 μM) for inducing cytotoxicity (Fig. 2D).

3.4. GSH forms the first line of defense against acrolein induced cytotoxicity

To determine whether GSH depletion corresponds with acrolein-induced cytotoxicity, GSH consumed was compared to the amount of LDH leakage. An association between cytotoxicity and intracellular GSH was found. It was observed that when GSH depletion exceeded 18–28 nmol/mg protein, cytotoxicity increased (Fig. 2E). A decrease in the amount of GSH of 18 nmol/mg protein is seen after exposure to 3 μM acrolein.

No toxicity was observed after addition of ⩽3 μM of acrolein and the results indicate that all the acrolein was captured by GSH (Fig. 2). At higher concentrations of acrolein only part of the acrolein was captured by GSH and toxicity was observed (Fig. 2). Together these results indicate that GSH forms the first line of defense against acrolein toxicity.

3.5. Adaptation to acrolein

To assess whether cells can adapt to acrolein, cells were pretreated with 3 μM acrolein 4 h prior to a second exposure of 10 μM acrolein. As also shown above, treatment of control cells to 3 μM of acrolein alone did not significantly increase cytotoxicity, whereas 10 μM of acrolein did significantly increased the observed cytotoxicity compared to cytotoxicity in control cells (Fig. 3A). In cells pretreated with 3 μM of acrolein, the cytotoxicity of 10 μM of acrolein was significantly diminished (Fig. 3A).

Exposure of BEAS-2B cells to 3 μM acrolein immediately decreased the amount of GSH (Fig. 3B). Furthermore, exposure to 10 μM of acrolein directly reduced intracellular GSH levels significantly more (Fig. 3B). Moreover, pretreatment with the non-toxic concentration of acrolein (3 μM) prevented a reduction of intracellular GSH by 10 μM of acrolein (Fig. 3B).

3.6. Acrolein-induced adaptation involves GSH induction

To assess whether cellular adaptation to acrolein involved GSH induction, cellular GSH levels and γGCS gene expression were analyzed at different times. Exposure of BEAS-2B cells to either 3 or 10 μM acrolein resulted in a rapid but transient reduction of intracellular GSH levels (Fig. 4A and B). Within approximately 3 h the amount of GSH returned to and increased significantly above baseline (Fig. 4A and B). Exposure to acrolein also resulted in an increase in γGCS mRNA expression (Fig. 4C and D).

4. Discussion

Hormesis is a theoretical concept in which it is suggested that exposure to a low dose of a chemical that is damaging at higher doses, induces an adaptive upgrade of the cellular protection [4,13]. If this theory is correct it has important ramifications for risk assessment and our perception of toxicity. This prompted us to investigate the hormesis concept and we used the α,β-unsaturated aldehyde acrolein.

Acrolein is a soft electrophile that prefers to react with soft nucleophiles, which in biological systems are predominantly thiols [8]. Acrolein directly induces cellular toxicity and is a model for electrophilic xenobiotics.

A cell is protected against electrophile-induced damage [14]. In BEAS-2B cells exposed to ⩽3 μM of acrolein, the acrolein is efficiently captured by GSH thus preventing cytotoxicity. We confirmed in the test tube that acrolein reacts with GSH within seconds having a stoichiometry of 1:1. Together this indicates that the nucleophilic tripeptide GSH is intracellularly the first line of defense against acrolein.

Within the cell, the capacity of GSH to quench acrolein appears to be limited. Upon raising the concentration of acrolein above 3 μM, GSH only captures part of the acrolein, which coincides with cytotoxicity apparent by LDH leakage. This observed acrolein-induced toxicity can be explained by adduction of critical thiol groups in proteins by the acrolein not captured by GSH [15]. This highlights the crucial role of GSH in the prevention of acrolein toxicity.

To study hormesis, the cells were first treated with a non-toxic concentration of 3 μM acrolein. This pretreatment appeared to reduce the cytotoxicity induced by a second exposure to a higher normally toxic concentration of acrolein (10 μM). This demonstrates that the pretreatment with the low dose induces

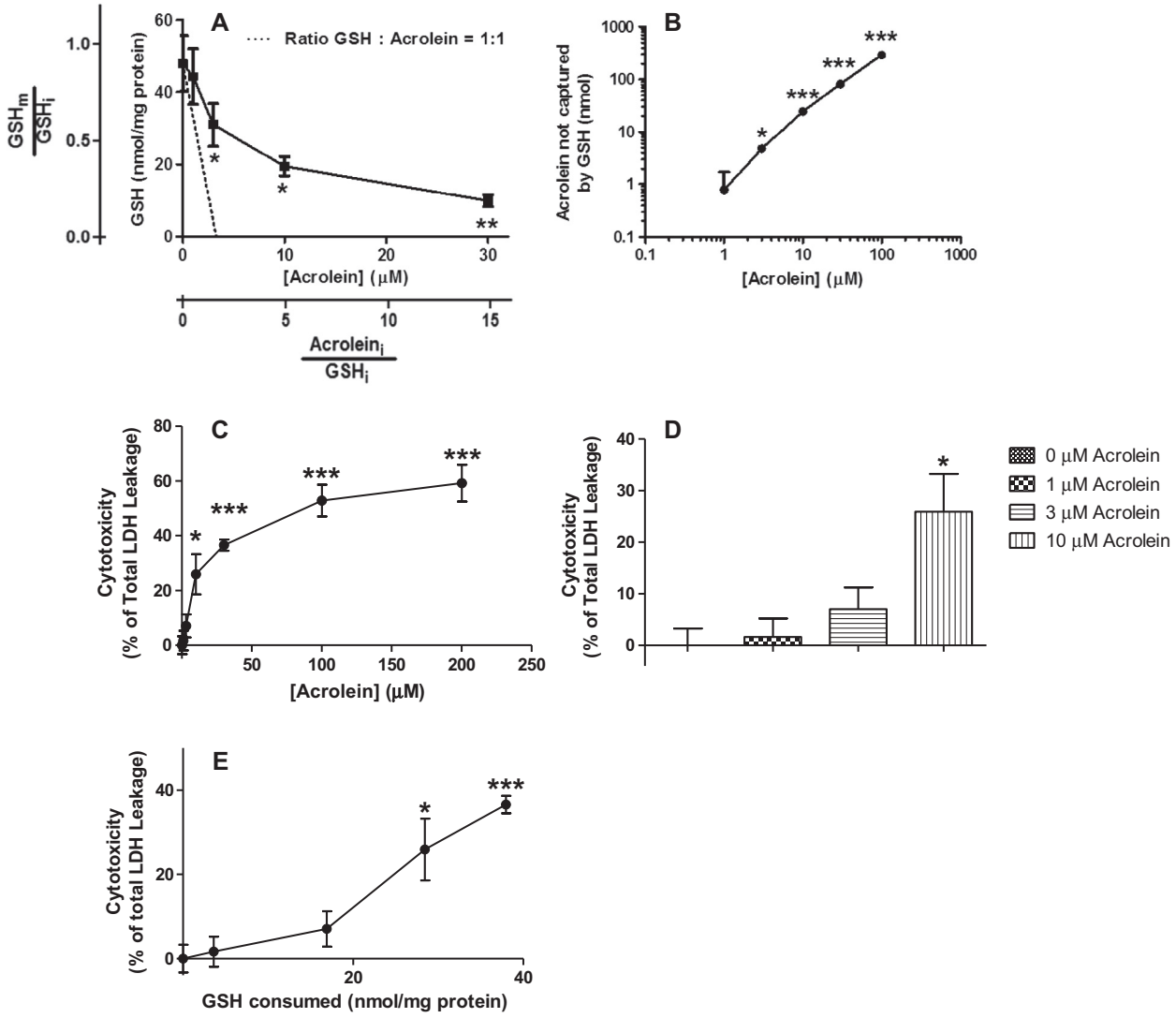

Fig. 2. The effect of acrolein on GSH levels and cell toxicity of BEAS-2B cells. (A) The intracellular GSH (nmol/mg protein) in BEAS-2B cells exposed to 0, 1, 3, 10 and 30 μM of acrolein. The dotted line represents the theoretical reduction in intracellular GSH due to acrolein based on the chemical reaction with a stoichiometry of 1:1. Both the initial amount of acrolein ($Acrolein_i$) relative to the initial intracellular amount of GSH (GSH_i) as well as the added concentration of acrolein are shown. Both the amount of GSH measured after the exposure (GSH_m) relative to the initial intracellular amount of GSH (GSH_i) as well as the amount of GSH (nmol/mg protein) are depicted. (B) The absolute amount of acrolein not reacted with intracellular GSH relative to the concentration of acrolein added. (C) Cytotoxicity in BEAS-2B cells exposed to 0, 1, 3, 10, 30, 100 and 200 μM of acrolein. Cytotoxicity is expressed as a percentage of LDH activity observed after cell lysis (addition of 3% Triton-X-100) and shown relative to cytotoxicity observed at exposure to 0 μM of acrolein. (D) Cytotoxicity in BEAS-2B cells exposed to 0, 1, 3 and 10 μM of acrolein. (E) Cytotoxicity plotted against the reduction in intracellular amount of GSH. $N = 4$ and data are shown as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to control (=0 μM of acrolein).

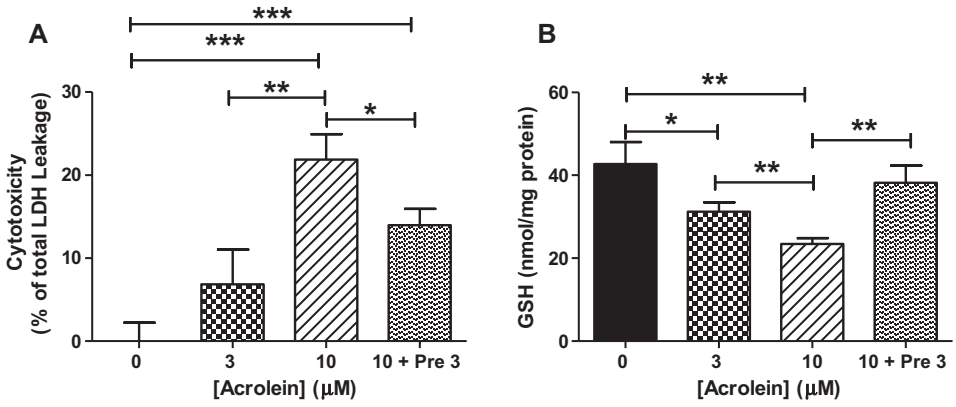

Fig. 3. Effect of pretreatment of BEAS-2B cells with 3 μM of acrolein on cytotoxicity (A) and GSH levels (B) induced by 10 μM of acrolein. $N \geqslant 6$ and data are shown as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to control (=0 μM of acrolein).

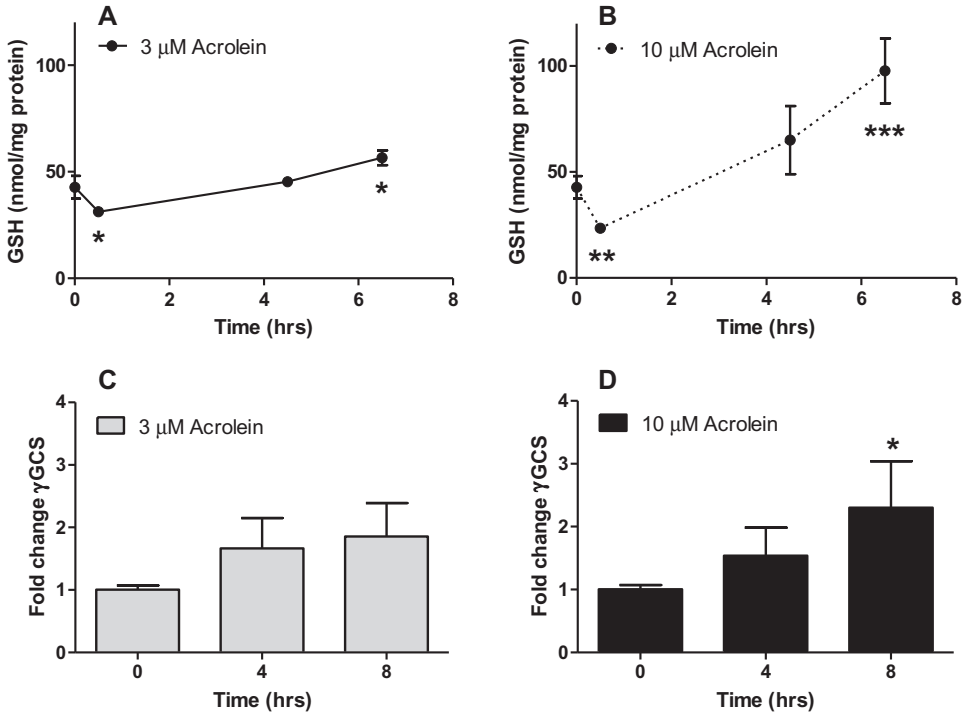

Fig. 4. The time course of GSH content in BEAS-2B cells after addition of 3 μM (A) or 10 μM (B) of acrolein. The expression γGCS in BEAS-2B cells exposed to 3 μM (C) and 10 μM (D) of acrolein. γGCS mRNA expression is shown relative to β-actin. $N \geqslant 3$ and data are shown as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to control ($T = 0$).

an adaptation. To further study this adaptive response of the cell to acrolein, we examined the molecular mechanism.

The crucial role of GSH in the prevention of acrolein toxicity prompted us to quantify GSH levels in the cell. After the expected initial decrease, an increase in the intracellular GSH concentration was found by the hormetic treatment with 3 μM acrolein. Upon a second exposure of 10 μM acrolein, the GSH concentration did not drop below control level. Apparently, acrolein pretreatment protects against the further depletion of GSH.

The hormetic exposure enforces the GSH defense. The rate limiting step in GSH synthesis is catalyzed by the enzyme γGCS [16]. Kelch-like ECH-associated protein 1 (Keap1) is known to be adducted by acrolein [17], which results in translocation of Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) to the nucleus [18], where it promotes the gene expression of antioxidant defense enzymes including γGCS [19]. Our results indeed show that γGCS expression increases.

Toxicity of acrolein appears to be alleviated by small hormetic dosages. This exemplifies the dynamics of cellular protection towards acrolein. This cellular flexibility is not acknowledged in risk assessment procedures. Nevertheless, hormesis does affect xenobiotic toxicity. A cell is triggered to enforce its protection before a specific hazard might become an imminent danger. The cell appears to be flexible in its defense and raises its specific shields when provoked. An adaptive strategy is far more economical and efficient than continuously wearing a "suit of armour" against all possible threats. Thus the challenge is to correctly incorporate hormesis in risk assessment to accurately assess toxicity. The paradigm of risk assessment is rigid, while in reality cells are flexible.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.03.081.

References

[1] Agency for Toxic Substances and Disease Registry. <http://www.atsdr.cdc.gov/substances/indexAZ.asp>, 2011
[2] U. Bandyopadhyay, D. Das, R.K. Banerjee, Reactive oxygen species: oxidative damage and pathogenesis, Curr. Sci. 77 (1999).
[3] What is health? The ability to adapt, Lancet 373 (2009) 781.
[4] E.J. Calabrese, L.A. Baldwin, Defining hormesis, Hum. Exp. Toxicol. 21 (2002) 91–97.
[5] J.F. Stevens, C.S. Maier, Acrolein: sources, metabolism, and biomolecular interactions relevant to human health and disease, Mol. Nutr. Food Res. 52 (2008) 7–25.
[6] G.R. Haenen, N.P. Vermeulen, J.N. Tai, J.N. Tai Tsoi, H.M. Ragetli, H. Timmerman, A. Bast, Activation of the microsomal glutathione-S-transferase and reduction of the glutathione dependent protection against lipid peroxidation by acrolein, Biochem. Pharmacol. 37 (1988) 1933–1938.
[7] M.J. Randall, M. Hristova, A. van der Vliet, Protein alkylation by the alpha, beta-unsaturated aldehyde acrolein. A reversible mechanism of electrophile signaling?, FEBS Lett 587 (2013) 3808–3814.
[8] H. Esterbauer, R.J. Schaur, H. Zollner, Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes, Free Radical Biol. Med. 11 (1991) 81–128.
[9] T.L. Ho, H.C. Ho, L.D. Hamilton, Biochemical significance of the hard and soft acids and bases principle, Chem. Biol. Interact. 23 (1978) 65–84.
[10] R. Nassini, S. Materazzi, E. Andre, L. Sartiani, G. Aldini, M. Trevisani, C. Carnini, D. Massi, P. Pedretti, M. Carini, E. Cerbai, D. Preti, G. Villetti, M. Civelli, G. Trevisan, C. Azzari, S. Stokesberry, L. Sadofsky, L. McGarvey, R. Patacchini, P. Geppetti, Acetaminophen, via its reactive metabolite N-acetyl-p-benzo-quinoneimine and transient receptor potential ankyrin-1 stimulation, causes neurogenic inflammation in the airways and other tissues in rodents, FASEB J. 24 (2010) 4904–4916.
[11] I. Rahman, A. Kode, S.K. Biswas, Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method, Nat. Protoc. 1 (2006) 3159–3165.
[12] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method, Methods 25 (2001) 402–408.
[13] M.P. Mattson, Hormesis defined, Ageing Res. Rev. 7 (2008) 1–7.
[14] M.L. Circu, T.Y. Aw, Reactive oxygen species, cellular redox systems, and apoptosis, Free Radical Biol. Med. 48 (2010) 749–762.
[15] J. Cai, A. Bhatnagar, W.M. Pierce Jr., Protein modification by acrolein: formation and stability of cysteine adducts, Chem. Res. Toxicol. 22 (2009) 708–716.

[16] I. Rahman, Regulation of glutathione in inflammation and chronic lung diseases, Mutat. Res. 579 (2005) 58–80.
[17] M. McMahon, D.J. Lamont, K.A. Beattie, J.D. Hayes, Keap1 perceives stress via three sensors for the endogenous signaling molecules nitric oxide, zinc, and alkenals, Proc. Natl. Acad. Sci. USA 107 (2010) 18838–18843.
[18] M.K. Kwak, T.W. Kensler, R.A. Casero Jr., Induction of phase 2 enzymes by serum oxidized polyamines through activation of Nrf2: effect of the polyamine metabolite acrolein, Biochem. Biophys. Res. Commun. 305 (2003) 662–670.
[19] H.Y. Cho, S.P. Reddy, S.R. Kleeberger, Nrf2 defends the lung from oxidative stress, Antioxid. Redox Signal. 8 (2006) 76–87.