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RESEARCH ARTICLE

The effects of changes in glutathione levels through exogenous agents on intracellular cysteine content and protein adduct formation in chronic alcohol-treated VL17A cells

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ABSTRACT

Alcohol-mediated liver injury is associated with changes in the level of the major cellular antioxidant glutathione (GSH). It is interesting to investigate if the changes in intracellular GSH level through exogenous agents affect the intracellular cysteine content and the protein adduct formation indicative of oxidative insult in chronic alcohol treated liver cells. In VL-17A cells treated with 2 mM *N*-acetyl cysteine (NAC) or 0.1 mM ursodeoxycholic acid (UDCA) plus 100 mM ethanol, an increase in cysteine concentration which was accompanied by decreases in hydroxynonenal (HNE) and glutathionylated protein adducts were observed. Pretreatment of 100 mM ethanol treated VL-17A cells with 0.4 mM buthionine sulfoximine (BSO) or 1 mM diethyl maleate (DEM) had opposite effects. Thus, altered GSH level through exogenous agents may either potentiate or ameliorate chronic alcohol-mediated protein adduct formation and change the cysteine level in chronic alcohol treated VL-17A cells. The gene expression of non-treated and ethanol-treated hepatocytes in 2 microarray datasets was also compared to locate differentially expressed genes involved in cysteine metabolism. The study demonstrates that increased protein adducts formation and changes in cysteine concentration occur under chronic alcohol condition in liver cells which may increase alcohol-mediated oxidative injury.

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Introduction

Glutathione (GSH) is ubiquitous in all animal cells, acts as a scavenger for reactive oxygen species (ROS) and a detoxification agent which conjugates with xenobiotics leading to their subsequent elimination from the cell (Mari & Cederbaum, 2000; Meister, 1995). Oxidative stress and changes in redox status characterize all forms of liver diseases (Yuan & Kaplowitz, 2009). One of the major mechanisms for the hepatotoxicity of ethanol involves induction of cytochrome P4502E1 (CYP2E1) and the subsequent formation of ROS and lipid peroxidation derivatives (Mari & Cederbaum, 2000). The relationship between GSH and alcohol-mediated liver injury varies, in some instances GSH, including the mitochondrial GSH is elevated (Bailey et al., 2001; Chandrasekaran et al., 2010), whereas in other studies, decreased GSH level are observed (Bansal et al., 2010; Waly et al., 2011).

One of the major determinants of the rate of GSH synthesis is the availability of cysteine (Lu, 1999; Tateishi et al., 1974). Cysteine differs from other amino acids since its sulfhydryl form, cysteine, is predominant inside the cell whereas its disulfide form, cystine is predominant outside the cell (Lu, 1999). GSH serves as a reservoir for cysteine because

extracellular cysteine is extremely unstable and rapidly auto-oxidizes to cystine, generating ROS in the process (Lu, 1999; Vina et al., 1983).

Cysteine is readily taken up by most if not all cells (Lu, 1999). Once inside the cell, the majority of cysteine is incorporated into GSH; and the rest is incorporated into protein or degraded into sulfate and taurine (Lu, 1999). For most cells, this mechanism provides a continuous source of cysteine. Thus, the gamma-glutamyl cycle allows the efficient utilization of GSH as cysteine storage (Lu, 1999).

N-Acetyl cysteine (NAC) contributes cysteine as a building block for GSH synthesis thus increasing GSH content (Estrela et al., 1983). Ursodeoxycholic acid (UDCA) increases the transcription of gamma glutamyl cysteine synthetase thus increasing the enzymatic efficiency (Mitsuyoshi et al., 1999). GSH depletion by buthionine sulfoximine (BSO) inhibits gamma-glutamylcysteine synthetase, the rate-limiting enzyme for GSH synthesis (Mari & Cederbaum, 2000). Diethyl maleate (DEM) depletes GSH through conjugation with GSH (Mitchell et al., 1983).

One of the aims of the study was to investigate the changes in intracellular cysteine concentration due to chronic alcohol and GSH supplementation or depletion. The study

also investigated the role of GSH as a protective mechanism against chronic alcohol mediated oxidative adduct formation. In order to study the above parameters, the present study employed the recombinant VL-17A cells over-expressing alcohol dehydrogenase (ADH) and CYP2E1 and HepG2 cells devoid of ADH and CYP2E1 expression pretreated with NAC or UDCA or BSO or DEM and then exposed to chronic alcohol (100 mM ethanol). Further the differential expression of genes involved in cysteine metabolism in alcoholic liver was also studied using microarray datasets (from Gene Expression Omnibus – GEO) and BRB array tool.

Materials and methods

Chemicals

Minimal essential medium (MEM), phosphate buffered saline (PBS), L-glutamine, and trypsin were obtained from Himedia, Mumbai, India. Fetal bovine serum, the antibiotics zeocin, geneticin, and gentamicin were obtained from Invitrogen (Carlsbad, CA). The antibody for reduced glutathione was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Other chemicals used were obtained from Sigma-Aldrich (St. Louis, MO). All chemicals were of the highest quality commercially available.

Cell culture and treatment

HepG2 cells, which do not express ADH and CYP2E1 and VL-17A cells, which are HepG2 cells constitutively expressing ADH and CYP2E1 were used for the study (Donohue et al., 2006). HepG2 cells were obtained from National Center for Cell Sciences, Pune, India. VL-17A cells were kindly provided by Dr. DL Clemens, University of Nebraska Medical Center and Veterans Affairs Medical Center, Nebraska USA. HepG2 and VL-17A cells were cultured in MEM containing 10% fetal bovine serum containing 2 mM L-glutamine and 50 µg/ml gentamicin in a humidified atmosphere in 5% CO₂ at 37 °C. Further, VL-17A cells were cultured in the presence of zeocin (selective for ADH) and geneticin (selective for CYP2E1) each at 400 µg/ml. Cells were plated for 24 h before being treated with the different chemicals at the indicated concentrations for the different experiments.

Experiments were performed with 2 mM NAC (Chandrasekaran et al., 2010) or 0.1 mM UDCA (Mitsuyoshi et al., 1999) as GSH supplementing agents or 0.4 mM BSO (Mari & Cederbaum, 2000) or 1 mM DEM (Hassan et al., 1992) as GSH-depleting agents. Both HepG2 and VL-17A cells were each divided into 10 experimental groups and the culture duration with or without the different agents was 72 h except otherwise indicated: (1) untreated cells in the first group served as the control; (2) cells treated with 100 mM ethanol (chronic alcohol); (3) cells treated with 2 mM NAC; (4) cells treated with 0.1 mM UDCA; (5) cells treated with 0.4 mM BSO; (6) cells treated with 1 mM DEM for 2 h; (7) cells treated with 2 mM NAC plus 100 mM ethanol; (8) cells treated with 0.1 mM UDCA plus 100 mM ethanol; (9) cells treated with 0.4 mM BSO plus 100 mM ethanol; and (10) cells treated with 1 mM DEM (for 2 h only) plus 100 mM ethanol.

Measurement of 4-hydroxynonenal adduct formation

Immunostaining for 4-hydroxynonenal (HNE) adduct formation was performed according to a method which was described previously (Chandrasekaran et al., 2010) in untreated liver cells, liver cells treated with 100 mM ethanol for 72 h, and liver cells treated with the different agents as indicated above with or without 100 mM ethanol for 72 h.

Detection of glutathionylated protein adduct formation

The formation of glutathionylated protein adducts was detected through immunostaining by a slight modification of the method described by Palanisamy et al. (2011) in untreated HepG2 and VL-17A cells, HepG2 and VL-17A cells treated with 100 mM ethanol for 72 h and HepG2 and VL-17A cells treated with the different agents as indicated above with or without 100 mM ethanol for 72 h. Briefly described, the liver cells were washed with PBS and fixed with methanol for 5 min at –10 °C and left air dried. After washing with PBS (3×), endogenous peroxidase was quenched by incubating the cells with 3% H₂O₂ (in PBS) for 10 min at room temperature. Further, after washing with PBS (2×), the fixed cells were treated with 5% non-fat milk for 1 h (blocking solution). The blocking solution was removed, cells were washed with PBS for 5 min and incubated for 3 h with mouse anti-GSH primary antibody (reacts with glutathione-protein complexes) at 1:400 dilution. After washing with PBS for 5 min (3×), cells were incubated with the secondary antibody conjugated with HRP at 1:200 dilution for 30 min. After washing with PBS for 5 min (2×), cells were incubated in 0.05% of diaminobenzidine for 15 min in dark, the staining developed was observed under light microscope.

Intracellular cysteine concentration

The intracellular cysteine concentration was measured by using a modified protocol of Gaitonde (1967) (Dominy et al., 2007; Gaitonde, 1967) in untreated liver cells, liver cells treated with 100 mM ethanol for 72 h, and liver cells treated with the different agents as indicated above with or without 100 mM ethanol for 72 h. Briefly, the method consisted of treating liver cells with 250 µl of 5% sulfosalicylic acid followed by centrifugation at 15,000 g for 15 min. The pH of the supernatant was adjusted to 8.3 using 10 N NaOH, then 5 mM dithiothreitol was added to it and the mixture was kept at room temperature for 15 min. Glacial acetic acid and acidified ninhydrin reagent (250 µl each) were added to 250 µl of the above solution, the reaction mixture was boiled at 100 °C for 10 min and the final volume was adjusted with 95% ethanol to 1 ml. The absorbance was measured at 560 nm and the cysteine concentration was quantified using a cysteine hydrochloride standard.

Data mining and meta-analysis

To study the expression of genes related to cysteine metabolism under ethanol (EtOH) treatment, 2 microarray datasets

from (GEO) under the accession numbers GSE10285 and GSE52644 (Tatsukawa et al., 2009; Yao et al., 2014) were retrieved. GSE10285 dataset contained the gene expression profiles of TG2+/+ (transglutaminase 2) and TG2−/− mice primary hepatocytes treated with or without 100 mM ethanol. GSE52644 dataset contained the gene expression data of C3H/HeN female mice (wild type or constitutive androstane receptor deficient) fed with Lieber's isocaloric diet containing water, or containing ethanol, or containing ethanol and ellagic acid or containing ethanol and *trans*-resveratrol for 1 week. The gene expression of control (non-treated) and ethanol-treated hepatocytes in both the datasets (GSE10285 & GSE52644) were compared using BRB-array tools.

Statistical analysis

The data represent the results of three experiments and is presented as mean ± SE. One-way ANOVA followed by Bonferroni post-hoc test was performed to analyze results between the different groups. $p < .05$ was considered to be statistically significant.

Results

Changes in HNE adduct formation in 100 mM alcohol-treated VL-17A cells with GSH supplementation or depletion

HNE adduct formation was not evident in untreated HepG2 cells (Figure 1). HepG2 cells treated with 100 mM ethanol were characterized by the complete absence of HNE

adduct formation. In contrast, 100 mM ethanol treated VL-17A cells exhibited extensive HNE adduct formation.

HepG2 cells treated with 2 mM NAC or 0.1 mM UDCA plus 100 mM ethanol exhibited a complete lack of HNE adduct formation. However, 2 mM NAC or 0.1 mM UDCA plus 100 mM ethanol-pretreated VL-17A cells were characterized by a minimal presence of HNE adduct formation. While 0.4 mM BSO or 1 mM DEM plus 100 mM ethanol-treated HepG2 cells did not exhibit HNE formation, 0.4 mM BSO or 1 mM DEM plus 100 mM ethanol-treated VL-17A cells exhibited increased HNE formation.

Changes in glutathionylated protein adduct formation in 100 mM alcohol treated VL-17A cells with GSH supplementation or depletion

Untreated HepG2 and VL-17A cells and HepG2 cells treated with 100 mM ethanol were characterized by a complete lack of glutathionylated protein adduct formation (Figure 2). VL-17A cells treated with 100 mM ethanol were characterized by a large number of cells showing dark brown staining due to glutathionylated protein adduct formation.

In HepG2 cells treated with 100 mM ethanol or 2 mM NAC or 0.1 mM UDCA or 0.4 mM BSO or 1 mM DEM plus 100 mM ethanol, glutathionylated protein adduct formation was not evident. Pretreatment of 100 mM ethanol-treated VL-17A cells with 2 mM NAC or 0.1 mM UDCA decreased the glutathionylated protein adduct formation when compared with 100 mM ethanol treated VL-17A cells, as observed through decrease in the intensity of the staining accompanied by increased cluster formation of cells. VL-17A cells treated with 0.4 mM BSO or 1 mM DEM plus 100 mM ethanol exhibited an

Figure 1. Effect of GSH supplementation or depletion on HNE-protein adduct formation. HepG2 and VL-17A cells were treated with 100 mM ethanol or 2 mM NAC or 0.1 mM UDCA or 0.4 mM BSO or 1 mM DEM plus 100 mM ethanol for 72 hours. HNE-protein adduct formation was detected by staining with anti-4-HNE (Santa Cruz Biotechnologies, USA) antibody. One representative experiment of three is shown. Bar: 20 μ m.

Figure 2. Effect of GSH supplementation or depletion on glutathionylated protein adduct formation in HepG2 and VL-17A cells. HepG2 and VL-17A cells were treated with 100 mM ethanol or 2 mM NAC or 0.1 mM UDCA or 0.4 mM BSO or 1 mM DEM plus 100 mM ethanol for 72 hours. Glutathionylation of protein was detected by staining liver cells with anti-GSH antibody. Bar: 20 μ m.

increase in glutathionylated protein adduct formation when compared with 100 mM ethanol-treated VL-17A cells along with a fewer number of cells.

Changes in cysteine concentration in 100 mM alcohol-treated VL-17A cells with GSH supplementation or depletion

Untreated HepG2 cells exhibited a 2.1-fold significantly higher intracellular cysteine concentration than the corresponding group of VL-17A cells (Figure 3). While HepG2 cells treated with 100 mM ethanol did not exhibit altered intracellular cysteine concentration, VL-17A cells treated with 100 mM ethanol exhibited a 1.1-fold significantly decreased intracellular cysteine concentration when compared with untreated VL-17A cells.

Treatment of HepG2 cells with 2 mM NAC or 0.1 mM UDCA plus 100 mM ethanol did not significantly change intracellular cysteine concentration when compared with 100 mM ethanol-treated HepG2 cells. VL-17A cells treated with 2 mM NAC or 0.1 mM UDCA plus 100 mM ethanol exhibited a 1.3-fold significantly higher intracellular cysteine concentration when compared with 100 mM ethanol-treated VL-17A cells.

Treatment of HepG2 cells with 0.4 mM BSO or 1 mM DEM plus 100 mM ethanol did not cause significant changes in intracellular cysteine concentration when compared with 100 mM ethanol treated HepG2 cells. Treatment of VL-17A cells with 0.4 mM BSO or 1 mM DEM plus 100 mM ethanol caused 1.2–1.5-fold significant decreases in intracellular

cysteine concentration when compared with 100 mM ethanol treated VL-17A cells

Identification of differentially expressed genes in 100 mM ethanol-treated murine hepatocytes and their putative roles in cysteine metabolism

The microarray datasets GSE10285 and GSE52644 were analyzed using BRB-array tools. In the GSE10285 dataset, 17,387 genes showed minimum spot intensity of 10 and a fold change of 1.5 in at least 20% of the arrays. Out of which, 2800 genes were significantly differentially expressed with the p value .05 in 100 mM ethanol treated hepatocytes. Ninety genes were significant at the nominal level 0.001 of the univariate test in which 11 genes were up-regulated and 79 genes were down-regulated in 100 mM ethanol-treated hepatocytes which are given as heatmaps (Figure 4 (A)).

Out of the differentially expressed genes (DEGs), *Got2* gene has been found to be associated with cysteine metabolism (Cresenzi et al., 2003). In a transgenic mouse model of Cystathionine β -synthase (cysteine synthase)-deficient homocystinuria, the expression of mitochondrial glutamic-oxaloacetic transaminase or aspartate aminotransferase (GOT2) was found to be repressed (Jiang et al., 2014).

GSE52644 dataset revealed 54 DEGs with the p value of 0.05. Comparison of the two datasets showed six genes in common which include *Stom*, *Sh3glb1*, *Hsd17b7*, *M6pr*, *Khdrbs1*, and *Ccne1* whose significance in cysteine metabolism is not currently known (Figure 4(B)).

Figure 3. Effect of GSH supplementation or depletion on intracellular cysteine concentration in HepG2 and VL-17A cells. HepG2 and VL-17A cells were treated with 100 mM ethanol or 2 mM NAC or 0.1 mM UDCA or 0.4 mM BSO or 1 mM DEM plus 100 mM ethanol for 72 hours. Intracellular cysteine concentration was assayed by ninhydrin method. Results are expressed as mean $\pm$ S.E of three experiments. * $p < .05$ compared with untreated HepG2 or VL-17A cells, $\phi p < .05$ compared with 100 mM ethanol treated HepG2 cells, $\neq p < .05$ compared with untreated HepG2 cells, # $p < .05$ compared with 100 mM ethanol treated HepG2 or VL-17A cells.

Discussion

In the present study, the effects of the modulation of GSH through exogenous antioxidants and GSH depleting agents on chronic alcohol mediated protein adduct formation in liver were investigated, suggesting alcohol-mediated oxidative damage. Cysteine serves as a crucial rate-limiting substrate for GSH synthesis (Lu, 1999; Tateishi et al., 1974). Further, in order to understand the mechanisms involved in changes in GSH homeostasis due to co-exposure of liver cells to chronic alcohol and exogenous GSH supplementation or depletion, the changes in intracellular cysteine concentration were studied.

All groups of HepG2 cells were characterized by a complete lack of HNE adduct formation. VL-17A cells treated with 100 mM ethanol exhibited HNE adduct formation which were decreased with pretreatment with 2 mM NAC or 0.1 mM UDCA. VL-17A cells treated with 0.4 mM BSO or 1 mM DEM plus 100 mM ethanol were characterized by the presence of extensive HNE adduct formation. As illustrated with the HNE adduct formation, the results decisively show that while exogenous GSH supplementation effectively decreases oxidative stress, a reverse phenomenon is observed with exogenous GSH depletion in 100 mM ethanol-treated VL-17A cells.

Deprivation of GSH in cells may lead to oxidative stress in cells since their redox equilibrium is altered and their ability to scavenge or detoxify ROS is impaired (Ghibelli et al., 1998). Depletion of GSH itself could lead to oxidative stress, independently of ROS generation (Ghibelli et al., 1998). Therefore, the exogenous addition of GSH ameliorates oxidative insult and the depletion of GSH exacerbates it further in 100 mM ethanol treated VL-17A cells, thus modulating alcohol-mediated oxidant injury.

Glutathionylated protein adducts which are formed in response to the cellular oxidative stress (Townsend, 2007) were not evident in untreated liver cells or liver cells treated

with the GSH supplementing or depleting agents. Glutathionylated protein adduct formation was not evident in 100 mM ethanol-treated HepG2 cells but the corresponding group of VL-17A cells was characterized by the presence of extensive glutathionylated protein adduct formation. While pretreatment of 100 mM ethanol treated VL-17A cells with 2 mM NAC or 0.1 mM UDCA decreased the glutathionylated protein adduct formation, 0.4 mM BSO or 1 mM DEM increased the glutathionylated protein adduct formation.

Protein S-glutathionylation occurs *in vivo* due to the thiol/disulfide exchange mechanism (Gilbert, 1995), which occurs when an oxidative attack leads to altered GSH/oxidized glutathione (GSSG) ratio and induces GSSG to bind to protein thiols (Pastore et al., 2003). Several important metabolic enzymes, transcription factors, and ubiquitin-activating enzymes are affected through protein glutathionylation (Pastore et al., 2003). Increased protein S-glutathionylation rate is associated with endoplasmic reticulum (ER) stress in some diseases and this overlap likely reflects signals emanating from the ER as it processes proteins that respond (e.g., by undergoing S-glutathionylation) to cellular redox conditions (Townsend, 2007).

HepG2 cells exhibited higher intracellular cysteine concentration than VL-17A cells, consistent with our observation of lower GSH level in HepG2 cells than VL-17A cells (data not shown). This implicates the utilization of cysteine in GSH synthesis, i.e. increased GSH level in VL-17A cells correlates with decreased cysteine level in VL-17A cells.

Since the cysteine concentration in 100 mM ethanol-treated HepG2 cells was not changed when compared with the untreated HepG2 cells, treatment of 100 mM ethanol-treated HepG2 cells with either 2 mM NAC or 0.1 mM UDCA or 0.4 mM BSO or 1 mM DEM did not change cysteine level further. However, 100 mM ethanol decreased cysteine concentration in VL-17A cells, a possible consequence of its

Figure 4. Identification of differentially expressed genes in 100 mM ethanol treated murine hepatocytes with putative roles in cysteine metabolism. (A) Heatmap of up-regulated and down-regulated genes in 100 mM ethanol treated group compared to control corresponding to the dataset GSE10285. (B) Venn diagram showing the number of differentially expressed genes in 100 mM ethanol treated group common in between the datasets GSE10285 and GSE52644.

utilization in GSH synthesis and, therefore, increased GSH level (data not shown).

While 2 mM NAC or 0.1 mM UDCA increased cysteine concentration in 100 mM ethanol treated VL-17A cells, 0.4 mM BSO or 1 mM DEM decreased the cysteine concentration. It is a well-established fact that both NAC and UDCA increase GSH synthesis through different mechanisms: NAC being a cysteine precursor (Estrela et al., 1983) and UDCA affecting the gamma glutamyl cysteine synthetase (Mitsuyoshi et al., 1999); therefore, the increase in cysteine with 2 mM NAC or 0.1 mM UDCA is not surprising. Cysteine concentration was

decreased with 0.4 mM BSO or 1 mM DEM plus 100 mM ethanol, implicating the lesser availability of cysteine for its utilization in GSH synthesis and in a larger context, impaired synthesis of other cellular proteins (Stipanuk et al., 2006).

An increase in intracellular cysteine level leads to increased GSH synthesis which is a consequence of increased saturation of glutamate–cysteine ligase with cysteine, and this contributes to the removal of excess cysteine (Stipanuk et al., 2006). A decrease in cysteine level also leads to increased glutamate–cysteine ligase activity and the increased capacity for GSH synthesis facilitates conservation

of cysteine in the form of GSH (Stipanuk et al., 2006). A very narrow range of cysteine levels ensures that the liver fulfills the two opposing homeostatic requirements (Stipanuk et al., 2006). On one hand, sufficiently high cysteine level is required to meet the needs of protein synthesis and the generation of other essential molecules that include GSH, coenzyme A, taurine, and inorganic sulfur (Stipanuk et al., 2006). On the other hand, cysteine concentrations must also be kept below the threshold of cytotoxicity (Stipanuk et al., 2006).

Since changes in cysteine concentration occurred due to 100 mM ethanol-treated VL-17A cells with or without GSH modulation, and cysteine being crucial for GSH synthesis, it was interesting to investigate if the genes expressed in alcoholic liver are involved in cysteine metabolism. Comparison of micro array data sets obtained from 100 mM ethanol treated murine hepatocytes revealed several differentially expressed genes in which the role of Got2 in cysteine metabolism is known till now. Aspartate aminotransferase catalyzes the transamination reaction of cysteine desulfuration pathway which redirects cysteine sulfinic acid towards pyruvate synthesis. In hepatocytes, the intracellular cysteine level is regulated either by incorporation to GSH or oxidation of cysteine to cysteine sulfinic acid (Cresenzi et al., 2003). The fate of oxidized cysteine, cysteine sulfinic acid, is to enter decarboxylation catalyzed by cysteine sulfinic acid decarboxylase for taurine production or to get transaminated by aspartate aminotransferase to form pyruvate (Stipanuk & Rotter, 1984; Stipanuk & Ueki, 2011). Based on our microarray data analysis, it could be predicted that the reduced expression of Got2 gene-encoded aspartate aminotransferase may enhance the ethanol-mediated taurine production. The reduced expression of Got2 gene in alcoholic liver favors the taurine synthesis in the hepatocytes as observed in a study involving rats administered with acute alcohol treatment (Jung et al., 2003). The authors concluded that ethanol depresses the cysteine availability for GSH synthesis not only by inhibiting the transsulfuration reactions but also by enhancing its irreversible catabolism to taurine (Jung et al., 2003). Further, taurine treatment has been shown to reverse hepatic steatosis induced by chronic alcoholic consumption (Kerai et al., 1999). Collectively the current study represents the importance of cysteine regulation in the development of alcoholic liver disease.

The results of the present study show that exogenous GSH supplementation with 2 mM NAC or 0.1 mM UDCA in VL-17A cells pretreated with 100 mM ethanol leads to increases in cysteine concentration. Further the NAC or UDCA plus ethanol-treated VL-17A cells showed decreased HNE and glutathionylated protein adducts. In contrast, GSH depletion through 0.4 mM BSO or 1 mM DEM in 100 mM ethanol-treated VL-17A cells decreased the cysteine level thus restricting its availability for GSH synthesis. Oxidative stress was higher as demonstrated through the HNE and glutathionylated protein adduct formation under chronic alcohol plus GSH-depleted VL-17A cells. Comparison of gene expression in 2 microarray data sets of non-treated and ethanol-treated hepatocytes revealed several differentially expressed genes, among which Got2 which encodes aspartate

aminotransferase has been found to be involved in cysteine metabolism till date. In conclusion, modulating GSH level with exogenous agents such as NAC, UDCA, BSO, or DEM in liver cells leads to changes in the oxidative environment which includes differential regulation of the various cellular processes affected by alcohol such as intracellular cysteine level and protein adduct formation.

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Disclosure statement

The authors declare that there are no conflicts of interest.

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