Mechanisms of Liver Injury.

III. Role of glutathione redox status in liver injury

Derick Han, Naoko Hanawa, Behnam Saberi, and Neil Kaplowitz

University of Southern California (USC) Research Center for Liver Diseases, USC-University of California-Los Angeles Research Center for Alcohol Liver and Pancreatic Diseases, Keck School of Medicine, USC, Los Angeles, California

Submitted 4 January 2006; accepted in final form 6 February 2006

Han, Derick, Naoko Hanawa, Behnam Saberi, and Neil Kaplowitz. Mechanisms of Liver Injury. III. Role of glutathione redox status in liver injury. Am J Physiol Gastrointest Liver Physiol 290: G1–G7, 2006.—GSH is the most abundant redox molecule in cells and thus the most important determinant of cellular redox status. Thiols in proteins can undergo a wide range of reversible redox modifications (e.g., S-glutathionylation, S-nitrosylation, and disulfide formation) during times of increased exposure to reactive oxygen and nitrogen species, which can affect protein activity. These reversible thiol modifications regulated by GSH may be nanoswitches to turn on and off proteins, similar to phosphorylation, in cells. In the cytoplasm, an altered redox state can activate (e.g., MAPKs and NF-E2-related factor-2) and inhibit (e.g., phosphatases and caspasases) proteins, whereas in the nucleus, redox alterations can inhibit DNA binding of transcription factors (e.g., NF-κB and activator protein-1). The consequences include the promotion of expression of antioxidant genes and alterations of hepatocyte survival as well as the balance between necrotic versus apoptotic cell death. Therefore, the understanding of the redox regulation of proteins may have important clinical ramifications in understanding the pathogenesis of liver diseases.

GSH and Cell Redox Status

Redox status has historically been used to describe the ratio of interconvertible reduced/oxidized forms of a molecule. The thiol group in the cysteine residue of GSH can become oxidized to form a disulfide (reaction 1):

$$2\text{GSH} \rightarrow \text{GSSG} + 2H^+ + 2e^-$$

(1)

To define a cellular redox state, the ratios of important and abundant redox molecules such as NAD+/NADH, NADP+/NADPH, and GSH/GSSG can be measured. However, because it is difficult to measure all linked redox couples in cells, a representative redox couple is generally measured. GSH/GSSG is the most important and commonly measured redox couple used to obtain an estimate of cellular redox state because 1) it is found at high levels in cells, far higher (≈100–10,000 greater) than most redox active compounds; and 2) the GSH/GSSG ratio is important in determining the redox status of proteins, thus influencing protein function and activity. An assessment of the GSH/GSSG ratio therefore provides a reliable estimation of cellular redox status in cells and is thus frequently measured as an indicator of oxidative stress.

Cellular GSH Levels and Compartmentation

GSH is synthesized by two enzymes: glutamate-cysteine ligase (GCL) and GSH synthetase, with the former catalyzing the rate-limiting step and being a point of feedback for GSH regulation (Fig. 1). A compartmentation of GSH occurs in cells, with mitochondria, the endoplasmic reticulum, and possibly the nucleus containing separate GSH pools. Mitochondrial GSH, which comprises about 10% of hepatic GSH, is generally considered more important than cytoplasmic GSH levels for cell survival. It is generally accepted that the GSH/GSSG ratio in cells is >100:1 under normal circumstances, but the redox ratio can shift to <4:1 during times of severe oxidative stress. The GSH/GSSG ratio is maintained by the enzyme GSSG reductase, which uses the reducing power of NADPH to convert GSSG to GSH. The GSH/GSSG ratio is thus ultimately tied to NADPH levels, which are determined by the energy status of the cell. Hepatocytes also export GSH through sinusoidal transport into plasma (Fig. 1) or into bile through canalicul transport.

Nernst Potential Determines Redox Status

The Nernst equation, which estimates the reduction potential between redox couples in an electrochemical cell, can be used to determine the redox potential of GSH in cells, thus providing an estimate of cellular redox status. The voltage of an electrochemical cell ($\Delta E$) can be determined by the following:

$$\Delta E = E_o - \frac{RT}{nF} \ln Q$$

where $E_o$ is the electromotive force under standard conditions,
MECHANISMS THAT ALTER GSH REDOX STATUS

GSH plays a key role in the detoxification of peroxides, reactive nitrogen species (RNS; e.g., peroxynitrite (ONOO⁻) and N₂O₃), and xenobiotic compounds (such as reactive electrophilic molecules) in cells (Fig. 1). Because the cellular GSH redox potential is affected by both GSH levels and the GSH/GSSG ratio, the cellular redox status can be altered by changes in the GSH/GSSG ratio. For example, a hepatocyte with just 1 mM GSH and a GSH/GSSG of 100:1 would have a potential of -330 mV, even though the GSH/GSSG ratio remained the same. This would suggest that cells with more GSH, such as hepatocytes, have a greater redox buffering capacity than cells with lower GSH levels, such as neurons.

GSH REDOX STATUS IN LIVER INJURY

MECHANISMS THAT ALTER GSH REDOX STATUS

GSH plays a key role in the detoxification of peroxides, reactive nitrogen species (RNS; e.g., peroxynitrite (ONOO⁻) and N₂O₃), and xenobiotic compounds (such as reactive electrophilic molecules) in cells (Fig. 1). Because the cellular GSH redox potential is affected by both GSH levels and the GSH/GSSG ratio, the cellular redox status can be altered by an increase in ROS or RNS generation (i.e., inflammation) that decreases the GSH/GSSG ratio and/or by the metabolism of drugs or other xenobiotic substances that deplete GSH. The GSH S-transferase family catalyzes the conjugation of GSH to many xenobiotic compounds (generally reactive electrophiles) to aid in their removal from cells. High levels of drugs and xenobiotic compounds that are detoxified by GSH can cause severe GSH depletion, and a dramatic shift in the cellular redox can occur. If mitochondrial GSH is depleted to levels below a certain threshold (-30-40% depletion), an increase in H₂O₂ release from mitochondria will occur, because GSH needed by GSH peroxidase to detoxify H₂O₂ becomes limiting. Thus a fall in GSH levels will lower the cellular redox status either directly, from a loss of GSH needed to maintain the redox buffer, or indirectly, by allowing increased exposure of cells to ROS that can oxidize cellular constituents because the GSH needed to detoxify peroxides becomes limiting. The maintenance of hepatic GSH and cellular redox status is therefore a dynamic process achieved by a balance between rates of GSH synthesis, GSH and GSSG efflux, GSH utilization by various enzymes, and NADPH levels (Fig. 1).
GSSG formation. Increases in H2O2 or ONOO− can also modulate protein function indirectly through involving guanylyl cyclase, by which NO affects cell signaling. Protein thiols may be an important mechanism, besides those −nitrosylate protein thiols and also GSH. The nitrosylation of mixed disulfide exchange and cause increased GSSG formation, which in turn can undergo further oxidation to sulfenic and sulfonic acids, which represent irreversible modifications. Recently, it has been shown that treatment of the mitochondrial protein aconitase with ONOO− results in sulfenic acid formation on cysteine residues and an inhibition of activity (9). ROS or RNS can also cause vicinal thiols or thiols from different proteins to undergo intra- or intermolecular disulfide bridge formation, respectively. Nitric oxide (NO), through the formation of higher nitrogen species such as N2O3, has been shown to S-nitrosylate protein thiols and also GSH. The nitrosylation of protein thiols may be an important mechanism, besides those involving guanylyl cyclase, by which NO affects cell signaling. ROS can also modulate protein function indirectly through GSSG formation. Increases in H2O2 or ONOO− in cells will cause increased GSSG formation, which in turn can undergo mixed disulfide exchange and S-glutathionylate proteins (Fig. 2). For this reason, GSSG is rapidly converted to GSH by GSSG reductase or actively transported out of cells. The localized environment of neighboring amino acids (pH, charge, etc.) determine the reactivity of the thiols to different stresses.

Changes in protein activity after thiol modifications. All the modifications to protein thiols outlined in Fig. 2 can potentially affect protein activity, with the degree depending on the importance of the cysteine residue in carrying out protein function. Many metabolic enzymes, kinases, phosphatases, and transcription factors contain critical cysteine residues that, if modified, can alter protein activity. Transcription factors such as NF-κB and c-Jun [activator protein-1 (AP-1)] contain critical cysteine residues that are needed for binding to DNA (18). The modification of thiols of cysteine residues can potentially affect binding to DNA and thus transcriptional activity. NF-κB has been shown to be S-glutathionylated and S-nitrosylated, both modifications causing a decrease in transcriptional activity. Some proteins may not contain cysteine residues important in protein function; however, modification of thiols may cause a conformational change that alters protein activity. Thus, like phosphorylation, the reversible modification of thiols, such as glutathionylation, may be a mechanism to turn on or off signaling pathways through modification of important cysteine residues or through the induction of conformational changes in the protein.

The exposure of cells to ROS is known to activate or inhibit many signaling cascades, including c-Jun NH2-terminal protein kinase (JNK), protein kinase C (PKC), and tyrosine kinase signaling. ROS such as H2O2 have been suggested to be second messengers in many signaling pathways in cells. However, to date, no receptor for H2O2 has been found in cells. H2O2 signaling may therefore depend on the oxidation of critical thiols in key signaling proteins, which results in a change of activity. Thiol modifications may also be a mechanism by which cells sense and regulate cellular redox status. The transcription factor NF-E2-related factor-2 (Nrf2) plays an essential role in the response to oxidative stress through the induction of antioxidant genes, including GCL, to upregulate GSH levels. Nrf2 is normally found in the cytoplasm bound to the inhibitory protein Kelch-like ECH-associated protein 1 (Keap 1). Thiols in Keap 1 act as redox sensors. Oxidation of thiols in Keap 1 will cause Nrf2 dissociation and translocation to the nucleus (5). Thus Keap 1 and Nfr2 act together to sense changes in cellular redox status, and, if redox alterations occur, genes necessary to restore cellular redox status are transcribed.

Regulation of protein redox status by GSH. The regulation of protein redox by GSH occurs through two mechanisms: 1) by buffering and protecting thiols on proteins from ROS and RNS and 2) by reversing disulfides bonds and nitrothiols that can occur during times of oxidative and nitrosative stress. GSH will intercept ROS and RNS, such as NO3•, that nitrosylate thiols of proteins. The reaction of N2O3 with GSH results in the formation of GSNO (Fig. 1). Because GSNO can potentially S-nitrosylate thiols in proteins, like GSSG, GSNO is rapidly reduced in cells by GSNO reductase.
The deglutathionylation of proteins can be accomplished directly by GSH, but the process is relatively slow. Therefore, in cells, deglutathionylation is catalyzed by glutaredoxin, a disulfide reductase [reaction 2 (15)]

\[
\text{Protein-S-SG + GSH} \rightarrow \text{Protein-SH + GSSG} \quad (2)
\]

Similarly, GSH can reduce intra- and interprotein disulfide cross-links of proteins, but this process is (again) relatively slow. In cells, the reduction of disulfides is catalyzed by thioredoxin (Trx). Trx contains vicinal thiols that react with and reduce disulfide cross-links (reaction 3)

\[
\text{Trx-S}_{2}\text{H}_{2} + \text{Protein-S-S-Protein} \rightarrow \text{Trx-S}_{2} + 2 \text{Protein-SH} \quad (3)
\]

The enzyme Trx reductase uses the reducing power of NADPH to regenerate Trx-S\textsubscript{2}H\textsubscript{2} from Trx-S\textsubscript{2} (15). Trx has been shown to be particularly important in keeping the thiols of NF-κB reduced in the nucleus so that it can effectively bind to DNA (14).

The high levels of GSH, along with redox-regulating enzymes [Trx, glutaredoxin, GSSG reductase, GSNO reductase, and other redox proteins (e.g., redox factor-1 and peroxiredoxins)] maintain proteins in cells in the reduced thiol form. This is in contrast to the extracellular environment in which GSH levels are low and proteins contain disulfides to maintain structural integrity (i.e., immunoglobins) in an oxidized environment. The cell spends a great deal of energy in the form of NADPH to keep the intracellular environment reduced.

**REDOX REGULATION OF APOPTOSIS AND NECROSIS**

Cell death is the crucial event leading to the clinical manifestation of hepatotoxicity from drugs, viruses, and alcohol. The redox alterations caused by ROS and RNS or agents that deplete GSH [e.g., diethylmaleate (DEM) and buthionine sulfoximine (BSO)] have been shown to induce apoptosis and necrosis in hepatocytes and other cells. This is because many proteins that modulate cell survival and death, such as NF-κB, JNK, PKC, and caspas, are redox regulated. Caspas, for example, are cysteine proteases that have been shown to be inactivated by nitrosylation or through the oxidation of thiols to disulfides (8, 18).

A common observation in many cell lines is that low levels of ROS such as H\textsubscript{2}O\textsubscript{2} can induce apoptosis, whereas higher levels lead to necrosis (1). Because critical cysteines on different proteins have different redox potentials, low levels of oxidants may be causing thiol oxidation in some proteins while not affecting other proteins. Thus low levels of oxidants may promote apoptosis by causing redox alterations that activate proapoptotic factors such as JNK and inhibit prosurvival proteins such as NF-κB (inhibiting DNA binding) without altering the redox status of proapoptotic caspas. In HL-60 cells, it has been estimated that a change in the redox potential by +72 mV promotes apoptosis (2). High levels of oxidants likely favor necrosis by inhibiting caspas needed to mediate apoptosis (8). Large redox changes caused by high amounts of oxidants such as H\textsubscript{2}O\textsubscript{2} or severe GSH depletion will cause necrosis in primary cultured hepatocytes, whereas modest redox changes have not been shown to promote apoptosis in hepatocytes.

However, changes in the intracellular environment by oxidants or GSH-depleting agents can regulate the response of hepatocytes to tumor necrosis factor-α (TNF) and Fas-induced apoptosis. Thus redox alterations in hepatocytes may be important in regulating the cell death pathways that mediate many liver pathologies.

**Alterations in Response to TNF by Redox Changes in Hepatocytes**

**TNF in liver diseases.** The cytokine TNF is an important regulator of inflammation in the liver. However, TNF is also believed to promote liver injury during inflammation. Several studies have shown that liver damage caused by exogenous agents such as carbon tetrachloride and chronic ethanol are dramatically decreased in mice lacking the TNF receptor. It is believed that sustained elevated levels of TNF promote apoptosis and necrosis in hepatocytes and, consequently, liver damage. TNF-induced apoptosis and necrosis of hepatocytes is believed to mediate, at least in part, liver damage during inflammation in alcoholic liver disease, liver ischemia, and liver toxicity caused by exogenous toxins, such as carbon tetrachloride.

TNF is a well-characterized antineoplastic agent that, upon exposure, triggers apoptosis and necrosis in many types of transformed cells. Hepatocytes, like most nontransformed cells, are resistant to TNF. The injection of TNF in vivo generally causes a proliferative response in the liver rather than a cytotoxic one. The resistance of hepatocytes to TNF has been attributed to the activation of NF-κB and transcription of NF-κB-dependent survival genes [such as inhibitor of apoptosis (IAP), inducible NO synthase (iNOS), and Bcl-X\textsubscript{L}] that protect hepatocytes from TNF. iNOS expression may be anti-apoptotic because NO production can lead to the nitrolysation and inactivation of caspas. The sensitization of hepatocytes to TNF can be achieved by interfering with NF-κB activation and/or transcription of NF-κB-dependent survival genes by treatment with agents such as actinomycin D (RNA synthesis inhibitor) (20).

The resistance of hepatocytes to the lethal actions of TNF has led to the question of how TNF promotes hepatocellular injury in various pathologies. Two possibilities can be considered: 1) TNF’s proinflammatory actions indirectly lead to hepatocellular injury and/or 2) TNF has a direct lethal effect on hepatocytes that have somehow been sensitized, possibly as a consequence of inflammation. Although interference with RNA or protein synthesis can sensitize cells to TNF-induced apoptosis in vitro and in vivo (blocking NF-κB survival gene expression), it is less clear as to what could be the mechanism of sensitization in liver diseases.

**Sensitization of hepatocytes to TNF-induced apoptosis by changes in cellular redox status.** Work done in our laboratory suggests that the redox alterations due to increased ROS and/or consequent GSH depletion that may occur during inflammation or caused by drug treatment may be an important mechanism in sensitizing hepatocytes to TNF-induced apoptosis (21, 22). Previous work from our laboratory has shown that GSH-depleting agents [e.g., acematinophen (APAP), phorone, and DEM] at doses that deplete mitochondrial GSH levels will induce necrosis in primary cultured hepatocytes. However, more modest doses of GSH-depleting agents that result mainly...
in cytoplasmic GSH depletion (cytoplasmic GSH depletion results in a minimal change in cell viability) were found to sensitize hepatocytes to TNF-induced apoptosis by inhibiting NF-κB-dependent survival gene expression (21, 22). Similarly, sublethal levels of H2O2 and redox-modulating agents such as diamide also sensitized primary cultured hepatocytes to TNF-induced apoptosis (10). The most redox-sensitive step in the pathway from IKK-mediated phosphorylation of IκB-α to the transactivation of NF-κB-dependent genes was the latter, presumably due to altered DNA binding of p50 and p65 subunits; this occurred after a modest selective depletion of cytosol GSH (40–50%). More severe, selective depletion of cytosol GSH (70–80%) or exposure to oxidants led to an inhibition of IκB-α phosphorylation (10, 21, 22). Thus redox alteration caused by GSH depletion or oxidants inhibits TNF-induced NF-κB-dependent expression of survival genes and thus promotes apoptosis.

On the basis of these findings, a dose relationship of the effect of H2O2 or decreases in GSH on hepatocyte apoptosis and necrosis can be proposed (Fig. 3). Moderate sublethal levels of H2O2 or depletion of cytoplasmic GSH cause cellular redox changes that interfere with NF-κB signaling, sensitizing hepatocytes to TNF-induced apoptosis. There is probably a redox range in which apoptosis can be initiated by TNF treatment of hepatocytes. High doses of H2O2 or substantial decreases in mitochondrial GSH ultimately cause necrosis in hepatocytes regardless of the presence of TNF through extensive oxidative damage that may overwhelm or even inhibit apoptotic signaling pathways, including caspase activity by oxidation of critical thiols. Therefore, massive necrosis caused by H2O2 or decreased GSH levels occurs after a certain critical redox threshold is passed, and, below this threshold, redox changes sensitize cells to TNF-induced apoptosis by inhibiting NF-κB activation and/or transactivation. Redox changes in hepatocytes caused by inflammation and drugs (i.e., APAP) may therefore be an important mechanism for sensitizing hepatocytes to TNF-induced apoptosis and promoting liver disease.

**REDOX REGULATION OF LIVER REGENERATION: ROLE OF TNF**

The plating of primary cultured hepatocytes under low density stimulates hepatocytes to undergo a shift from the G0 to G1 phase in the cell cycle and an increase in GSH levels. The increase in GSH is a result of increase synthesis of GCL in hepatocytes. Similarly, GSH levels after partial hepatectomy doubled due to increased GCL expression and activity (16). This suggests that redox changes may be important in regulating hepatocyte division. The increased GSH in the liver after partial hepatectomy was not associated with a change in the GSH/GSSG ratio. With the use of the Nernst equation, the cellular redox of liver cells goes from −330 to −340 mV after partial hepatectomy, assuming a GSH level of 10 mM and no changes in the GSH/GSSG ratio. This suggests a strong reducing environment is necessary for cell replication. In support of this notion is the observation that liver regeneration was inhibited by GSH depletion. The alteration in redox potential may be a common feature in dividing cells, because ROS have been suggested to modulate the cell cycle in many cell lines. Because TNF is secreted and NF-κB activation is essential for hepatocyte survival after partial hepatectomy (4), an increased reducing environment caused by increased GSH may be generated to ensure proper NF-κB activity and hepatocyte survival.

**MODULATION OF FAS-INDUCED APOPTOSIS BY REDOX ALTERATIONS**

Fas ligand belongs to the TNF/nerve growth factor superfamily that transduces apoptotic signals through activation of caspases. Treatment of mice with agonistic anti-Fas antibody triggers apoptosis in hepatocytes and liver failure. The notion that Fas signaling could be altered by redox changes arose from the observation that acute GSH depletion by phorone treatment prevented apoptosis induced by anti-Fas treatment (13). GSH esters but not antioxidants were found to restore the susceptibility of GSH-depleted mice against Fas-mediated apoptosis, suggesting that redox changes were important in modulating Fas signaling. The inhibition of apoptosis caused by GSH depletion by phorone was likely due to the redox modulation of caspase 3, which has thiol essential for mediating apoptosis. However, Fas signaling is complex and involves many redox-regulated proteins. Other researchers have observed that GSH depletion from feeding animals a sulfur amino acid-deficient diet (a slow, prolonged GSH depletion) exacerbated Fas-mediated apoptosis through increased p53 and Bax expression (11). This suggests that acute and prolonged redox changes may affect different redox proteins and thus may have varying effects on cell signaling pathways.

**EFFECT OF REDOX CHANGES IN VARIOUS LIVER INJURY MODELS**

Liver injury can be studied using various toxins such as galactosamine (Gal) cotreated with either TNF or LPS, concanavalin A, LPS alone, and α-amanitin. Hentze et al. (12) observed that liver injury caused by the above agents could be inhibited by acute GSH depletion caused by phorone treatment (12). Because many of these toxins mediate liver injury through mechanisms independent of caspase 3, the results...
suggest that other signal proteins, besides caspases, that mediate liver injury are regulated by redox changes. Another factor in vivo studies is different consequences of inhibition of the NF-κB pathway in different cell types; whereas GSH depletion inhibits survival gene expression, sensitizing cells to TNF-induced apoptosis in hepatocytes, inhibition of NF-κB in nonparenchymal cells may exert an anti-inflammatory effect by inhibiting the expression of cytokines, chemokines, and adhesion molecules (17). Although an acute shift from a reduced to a more oxidized state protected the liver from various toxins, the protection is probably not universal. Xu et al. (25) observed that GSH depletion with BSO treatment (a slower and prolonged means of GSH depletion) increased liver damage caused by Gal/LPS. This again points out that acute and long-term redox changes may affect cell signaling pathways differently and switch on and off different proteins.

REDOX CHANGES IN APAP-INDUCED LIVER INJURY AND JNK ACTIVATION

The importance of GSH in hepatocyte survival is demonstrated in APAP-induced liver injury. The cytochrome P-450 system in hepatocytes metabolizes APAP to N-acetyl-p-benzoquinone (NAPQI), which is detoxified by conjugation with GSH. An overdose of APAP causes severe GSH depletion, ROS generation, and covalent binding of NAPQI to protein, resulting in cell death. Nrf2, through redox alterations, is activated after APAP treatment so that GSH synthesis can be increased in hepatocytes. N-acetylcycteine, which can replenish GSH, is effective in treating patients with APAP-induced liver injury. Not surprisingly, GSH depletion by phosphorine or other agents potentiated APAP-induced liver injury in vivo.

Recent studies have suggested that cell death caused by GSH depletion and ROS generation is not sufficient for APAP-induced hepatotoxicity. Indeed, activation of redox-regulated proteins such as JNK may be necessary to mediate hepatocyte death caused by APAP (7). JNK and upstream kinases such as apoptosis signal-regulating kinase-1 are inhibited by Trx and GSH transferase subunits (π and others) (23). ROS and GSSG can oxidize these inhibitors and release active kinases. In addition, protein tyrosine phosphatases, including JNK phosphatase, have active site thiolate cysteines, which readily undergo oxidation by H₂O₂ to sulfenic acids (which inhibit these enzymes, thus favoring the persistence of activated phosphokinases). The understanding of how redox alterations activate JNK and other signaling proteins is an important question in understanding APAP-induced liver injury.

MITOCHONDRIA REDOX CHANGES IN LIVER DISEASES

Mitochondrial GSH is much more important for hepatocyte survival than cytoplasmic GSH. The mitochondrial redox status has profound effects on the cellular redox status as a whole, because the depletion of mitochondrial GSH can cause increased release of H₂O₂ from the mitochondrial matrix that can oxidize cytoplasmic proteins and affect cell signaling. Chronic ethanol treatment and hepatitis C infection (19) have been shown to cause mitochondrial GSH depletion. Chronic ethanol treatment is believed to selectively deplete mitochondrial GSH by decreasing GSH transporter activity through ethanol-induced changes in inner membrane fluidity due to cholesterol accumulation (6). In addition, the change in the mitochondrial redox potential caused by mitochondrial GSH depletion may also be important in regulating mitochondrial permeability transition (MPT). MPT is the opening of the mitochondrial megapore [composed of voltage-dependent anion channels, adenine nucleotide transporter (ANT), and other proteins] that may be important in necrosis and apoptosis, because it results in the loss of bioenergetics and release of prosapoptotic proteins such as cytochrome c. ANT and other components of the megapore are believed to have thiols that are important in regulating pore opening and closing (3). Disulfide formation on ANT will favor pore opening and a consequent loss of mitochondrial function. The redox regulation of MPT may be important in mediating APAP-induced liver injury. APAP treatment will trigger MPT in hepatocytes likely through redox alterations in ANT and other mitochondrial proteins, which mediates hepatocyte necrosis. Mitochondrial GSH changes may have profound effects on cellular redox status through modulating ROS generation, as well as necrosis and apoptosis through the regulation of MPT, and thus may be an important mechanism of mediating liver injury.

PERSPECTIVES

GSH is the most abundant redox molecule in cells and thus the most important determinant of cellular redox status. Both the levels of GSH and GSH/GSSG ratio determine the redox status of proteins in cells. Thiols in proteins can undergo a wide range of reversible redox modifications during times of increased exposure to ROS and RNS, which can affect protein activity. These reversible thiol modifications regulated by GSH may be nanoswitches to turn on and off proteins, similar to phosphorylation, in cells. In the cytoplasm, an altered redox state can activate (MAPKs, PKC, and Nrf-2) and inhibit (protein phosphatases, caspases, and Ikk) proteins, whereas in the nucleus redox alterations can inhibit DNA binding of transcription factors (NF-κB and AP-1). The consequences include the promotion of expression of antioxidant genes and alterations of cell survival as well as the balance between necrotic versus apoptotic cell death. Many liver diseases involve redox changes caused by GSH depletion or increased ROS and RNS generation. Therefore, understanding the redox regulation of proteins may have important clinical ramifications in understanding the pathogenesis of liver diseases and developing therapeutic approaches.

GRANTS

This work was supported by National Institutes of Health Grants RO1-DK-067215, P30-DK-48522, and P50-AA-11999.

REFERENCES


