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OXIDATIVE STRESS, TOXICOLOGY, AND PHARMACOLOGY OF CYP2E1*

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■ **Abstract** This review describes some of the biochemical and toxicological properties of CYP2E1, especially as it relates to alcohol metabolism and toxicity and the establishment of human hepatoma HepG2 cell lines that overexpress human CYP2E1. Ethanol, polyunsaturated fatty acids, and iron were found to be cytotoxic in HepG2 cells that overexpress CYP2E1. GSH appears to be essential in protecting HepG2 cells against the CYP2E1-dependent cytotoxicity, and GSH levels were elevated owing to a twofold increase in activity and expression of glutamate cysteine ligase. We suggest that this up-regulation of GSH synthesis was an adaptive response to attenuate CYP2E1-dependent oxidative stress and toxicity. Induction of a state of oxidative stress appears to play a central role in the CYP2E1-dependent cytotoxicity. Mitochondrial membrane potential decreased in the CYP2E1-expressing HepG2 cells, and this decrease shared similar characteristics with the developing toxicity. Alcohol-dependent liver injury is likely to be a multifactorial process involving several mechanisms. We believe that the linkage between CYP2E1-dependent oxidative stress, mitochondrial injury, and GSH homeostasis contribute to the toxic actions of ethanol on the liver.

INTRODUCTION

Oxidative stress is defined as a disturbance in the prooxidant/antioxidant balance in favor of the former, potentially leading to damage, and has been suggested to be a relevant factor in different pathological conditions (1). ROS have been implicated in many of the major diseases that plague humans, and it is becoming hard-pressed

*Abbreviations: AA, arachidonic acid; BSO, buthionine 1-sulfoximine; GCL; glutamate cysteine ligase; GSH, glutathione; H_2O_2, hydrogen peroxide; LDH, lactate dehydrogenase; MPT, mitochondrial permeability transition; NADPH, nicotinamide adenine dinucleotide phosphate; NASH, nonalcoholic steatohepatitis; NTA, nitrilotriacetate; O_2^-, superoxide anion radical; OH, hydroxyl radical; PI, propidium iodide; PT, permeability transition; ROS, reactive oxygen species.

to find a disease not involving, at least in part, ROS. A partial list includes the toxicity of O_2 itself; hyperbaric O_2; ischemia-reperfusion injury; cardiovascular diseases; atherosclerosis; carcinogenesis; diabetes; neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease; toxicity of heavy metals, e.g., iron; asbestos injury; radiation injury; vitamin deficiency; drug (e.g., redox cycling agents) toxicity; aging, inflammation; smoke toxicity; emphysema; and cataracts (2, 3).

The ability of acute and chronic ethanol treatment to increase production of ROS and enhance peroxidation of lipids, protein, and DNA has been demonstrated in a variety of systems, cells, and species, including humans. Despite the tremendous growth in understanding alcohol metabolism and actions, the mechanism(s) by which alcohol causes cell injury are still not clear. A variety of leading mechanisms have been briefly summarized (4–6), and it is likely that many of them ultimately converge as they reflect a spectrum of the organism's response to the myriad of direct and indirect actions of alcohol. A major mechanism that is a focus of considerable research is the role of lipid peroxidation and oxidative stress in alcohol toxicity. What is the evidence that ethanol-induced oxidative stress plays a role in cell injury? Although many studies have shown increases in lipid peroxidation or protein carbonyl formation by alcohol, it is not always clear if these are causes of or consequences of the alcohol-induced tissue injury. Nevertheless, there are many studies that show that administration of antioxidants or iron chelators or GSH-replenishing agents can prevent or ameliorate the toxic actions of alcohol. The most convincing data that oxidative stress contributes to alcohol-induced liver injury come from the studies using the intragastric infusion model of alcohol administration. In these studies, alcohol-induced liver injury was associated with enhanced lipid peroxidation, protein carbonyl formation, formation of the 1-hydroxyethyl radical, and decreases in hepatic antioxidant defense, e.g., GSH (7–11). Alcohol plus polyunsaturated, but not saturated, fat was required for the injury to occur. Addition of iron, known to generate $^{\bullet}OH$ and promote oxidative stress, to these diets exacerbated the liver injury (12). Importantly, addition of antioxidants, such as vitamin E, ebselen, superoxide dismutase, and GSH precursors, prevented the alcohol-induced liver injury.

In addition to these in vivo studies, in vitro studies with hepatocytes also showed that ethanol can produce oxidative stress and hepatocyte toxicity. Studies with isolated hepatocytes from control rats or chronically ethanol-fed rats indicated that ethanol metabolism via alcohol dehydrogenase results in an increase in ROS production, hepatocyte injury, and apoptosis, reactions blocked by antioxidants (13, 14). Whereas most ethanol is oxidized by alcohol dehydrogenase, CYP2E1 assumes a more important role in ethanol oxidation at elevated concentrations of ethanol and after chronic consumption of ethanol (15). Besides being present in the liver, small amounts of CYP2E1 can also be found in nonhepatic tissues, such as brain, kidney, and gastrointestinal tract (16). Major interest in CYP2E1 reflects the ability of this enzyme to oxidize ethanol; to generate reactive products from ethanol oxidation, e.g., acetaldehyde and the 1-hydroxyethyl radical; to activate various agents (CCl_4, acetaminophen, benzene, halothane, halogenated alkanes,

alcohol) to reactive products; to generate reactive oxygen species; and to be "induced" by ethanol (as well as several low-molecular-weight agents) (reviewed in 6, 16–20).

Understanding the biochemical and toxicological properties of CYP2E1 is important for many reasons, even in addition to its role in contributing to alcohol-induced liver injury because CYP2E1 is induced under a variety of pathophysiological conditions.

Diabetes: Diabetes has been reported to increase the expression of CYP2E1 mRNA and protein several-fold (21). Oxidative damage plays an important role in the pathophysiology of diabetes (22). CYP2E1 has been implicated in the generation of tissue-damaging hydroxyl radical in patients with diabetes (23).

Obesity: CYP2E1 has been reported to be elevated in chronically obese, overfed rats (24) and in rats fed a high-fat diet (25).

Fasting: In animals, levels of CYP2E1 were increased by fasting (26, 27) and prolonged starvation (28).

Cancer: Increased activity of CYP2E1 has been associated with increased risk of cancer through an increased production of ROS and enhanced activation of a variety of procarcinogens (29).

NASH: NASH is a condition characterized by hepatomegaly, elevated serum aminotransferase levels, and a histologic picture similar to alcoholic hepatitis in the absence of alcohol abuse (30). Hepatic CYP2E1 was increased in humans with NASH (31). Oxidative stress and lipid peroxidation is one of the critical factors involved in the genesis and probably the progression of NASH (32). In a mouse model of NASH, hepatic CYP2E1 was up-regulated, and this was associated with a dramatic increase in total lipid peroxide levels that were substantially inhibited by anti-CYP2E1 antibody (33).

Levels of CYP2E1 are influenced by a variety of hormones, e.g., hypophysectomy and T3 increase CYP2E1 protein and mRNA levels, whereas insulin lowers these levels (34, 35). Besides ethanol, CYP2E1 can be induced by drugs, such as isoniazid; hydrocarbons, such as trichloroethylene; benzene; chloroform; and solvents, such as DMSO, acetone, and pyridine (17, 18). Restriction fragment length polymorphisms for CYP2E1 have been described; some of these polymorphisms may be associated with or be risk factors for various forms of cancers, e.g., esophageal, lung, and breast cancer, and cigarette smoking–related hepatocarcinogenicity (36–38). Low levels of CYP2E1 are expressed in human fetal liver and prenatal brain, leading to suggestions that CYP2E1-derived ROS could play a key role in the fetal alcohol syndrome (39, 40).

In view of the induction of CYP2E1 under a variety of conditions and by many chemicals, further understanding the biochemical and toxicological properties of CYP2E1 and how levels of CYP2E1 are regulated within the cell is important. An approach that our laboratory has utilized to try to understand basic effects and actions of CYP2E1 is to establish HepG2 cell lines that constitutively express

human CYP2E1. Results obtained with these cell lines form the basis of this review.

Microsomes represent a potential source of ROS via cytochrome P450: The NADPH-dependent reduction of O_2 by cytochrome P450 in the presence and absence of substrate to superoxide anion radical and to hydrogen peroxide is well documented for all forms of the enzyme (18). Hydrogen peroxide and superoxide production occur either by decomposition of the ferrous-dioxygen complex (oxygenated P450) or by release from the peroxy-ferric intermediate (peroxy P450) formed during the catalytic cycle (41). ROS generation may vary considerably depending on the P450, the absence or presence of substrate, the nature of the substrate, and the action of cytochrome b5. Compared with other forms of P450, CYP2E1 exhibits a higher rate of oxidase activity when purified (42) and microsomes from animals pretreated with inducers of CYP2E1 also exhibit much greater rates of NADPH oxidation than microsomes from untreated animals. Enhanced oxidase activity would result in the increased production of O_2^- and H_2O_2, which, in the presence of chelated iron, can produce reactive hydroxyl radical (43, 44). The increased capacity to produce active oxygen species is manifested by an increased rate of microsomal lipid peroxidation by microsomes or liposomes enriched in CYP2E1 (45, 46). Antibody to CYP2E1 partially inhibited microsomal peroxide production while almost completely inhibiting NADPH-dependent lipid peroxidation (45), thus showing that CYP2E1 is a fundamental source of microsomal ROS.

Studies were carried out comparing microsomal interaction with iron and ROS production by microsomes from rats fed the Lieber-DeCarli ethanol diet for four weeks and from pair-fed controls. Under these conditions, CYP2E1 levels are elevated about three- to fivefold. The microsomes from the chronic ethanol-fed rats were about two- to threefold more reactive in generating superoxide radical and H_2O_2 and, in the presence of ferric complexes, in generating hydroxyl radical and undergoing lipid peroxidation. Microsomes from the ethanol-fed rats were twice as reactive as pair-fed- or chow-fed-derived microsomes in catalyzing inactivation of added enzymes, such as lactic dehydrogenase, alcohol dehydrogenase, or pyruvic kinase, in the presence of ferric-ATP or ferric-citrate. Similarly, microsomes from the ethanol-fed rats were more reactive in catalyzing DNA strand cleavage when plasmid DNA was added to the microsomal incubation system. In all the above systems, the enhanced effectiveness of microsomes from the ethanol-fed rats was prevented by addition of chemical inhibitors of CYP2E1 and by polyclonal antibody raised against CYP2E1 purified from pyrazole-treated rats (47), validating that the increased activity in the microsomes was due to CYP2E1 (43, 48–51). We suggest that one mechanism by which iron potentiates alcohol hepatotoxicity may be related to production of a state of oxidative stress and enhanced lipid peroxidation as a consequence of the interaction of iron with CYP2E1-derived ROS.

What is the functional and toxicological significance of the above studies that show that microsomes enriched in CYP2E1 are more reactive in producing ROS? To understand basic effects and actions of CYP2E1, our laboratory established

cell lines that constitutively express the human CYP2E1. HepG2 cells are a human hepatoblastoma cell line, which maintain several liver functions but do not express significant amounts of CYP2E1. HepG2 cells that overexpress CYP2E1 were established either by retroviral infection methods (MV2E1–9 cells, or E9 cells) or by plasmid transfection methods (E47 cells). Microsomes from transduced clones (E9 cells) produced a band in Western blots at 54 kDa migrating at the same position as the P4502E1 band from human liver microsomes and were catalytically active with effective substrates for rodent and human CYP2E1 (para-nitro phenol, aniline, dimethyl nitrosamine, and ethanol). Microsomes from transduced clones produced superoxide radicals, with rates one order of magnitude higher than that for control microsomes. The rate of H_2O_2 production was threefold greater. Microsomal lipid peroxidation catalyzed by low concentrations of Fe-ADP using NADPH as cofactor was higher in microsomes from CYP2E1-expressing cells (52). Thus, microsomal overexpression of active CYP2E1 was able to increase microsomal production of ROS.

Overall cellular generation of ROS was also increased by overexpression of CYP2E1. CYP2E1-expressing cells (E47 cells), in the absence of added toxins, showed a 40%–50% increase in intracellular production of reactive oxygen species, as assessed by oxidation of dichlorofluorescein diacetate in intact cells (53), and increased lipid peroxidation (increased levels of end products of lipid peroxidation, such as malondialdehyde plus 4-hydroxynonenal) compared with controls (54). Negative effects in cell proliferation (but not in viability) were detected in E47 cells, with doubling times of 30 h versus 21 h in controls. Growth inhibition in E47 cells was blocked by antisense inhibition of CYP2E1 and by vitamin E. Levels of ATP were 30% lower in E47 cells because of damage to complex I, which was prevented by vitamin E (54). The results suggest that CYP2E1 overexpression by itself produces increased oxidative stress and deleterious effects at the cellular proliferation level, probably caused by mitochondrial damage.

Treatments such as depletion of glutathione with L-buthionine sulfoximine (BSO) (54), addition of oxidizable substrates such as polyunsaturated fatty acids (AA) (55), redox-active metals [Fe(III) + BSO (56), Fe(III) + AA (57)], and ethanol (58) were more toxic to CYP2E1-expressing cells than to control cells. Evidence that increased production of ROS was a cause of the increased cytotoxicity was given by the following: (*a*) Toxicity in all these treatments was associated with increased peroxidation of cellular lipids, and exogenous exposure to antioxidants inhibited both lipid peroxidation and toxicity. Moreover, overexpression of catalase in the cytosolic or mitochondrial compartment inhibited toxicity and lipid peroxidation caused by GSH depletion (59) and Fe+AA (60) in CYP2E1-overexpressing cells. (*b*) The generation of ROS, as assessed by intracellular DCFH-DA oxidation, increased before the loss of viability [i.e., before the uptake of PI] after exposure to Fe+AA (61), and this increase together with the toxicity was inhibited by superoxide dismutase mimetics (62).

Two types of cell death have been distinguished by morphological features: apoptosis and necrosis. Our studies with CYP2E1-expressing cells show that both

modes of cell death can occur depending on the nature of the insult. Depletion of GSH produced a mixed type of cell death in E47 cells, involving apoptosis and necrosis. This conclusion is supported by the following: (*a*) E47 cells treated with BSO, double stained with annexin V and PI and analyzed by flow cytometry, showed an increase in the annexin V positive/PI negative population of cells—indicative of apoptosis—but also of the annexin V positive/PI positive population of cells—indicative of necrosis and late apoptosis (59); (*b*) trypan blue uptake increased with time in E47 cells incubated with BSO, indicative of early plasma membrane permeabilization, a necrotic feature (63); and (*c*) DNA laddering (54) and caspase 3 activation (63), characteristic of apoptotic processes, were also detected in CYP2E1-expressing HepG2 cells treated with BSO. Incubation of CYP2E1 overexpressing cells with arachidonic acid showed characteristic apoptotic features, such as DNA laddering (55), increased release of cytochrome c, and caspase 3 activity (64), but also increased early trypan blue uptake, indicative of plasma membrane permeabilization, a necrotic feature (65). Toxicity in E47 cells exposed to Fe+AA was mainly necrotic in nature, based on morphology (cell swelling, vacuolization of cytoplasm), early disruption of plasma membrane integrity (leakage of LDH), early depletion of ATP levels, and nonsignificant DNA degradation at early periods (3–6 h), where toxicity was already apparent (57). These observations suggest that massive oxidative stress, such as the combination of Fe+AA+CYP2E1, induces necrosis, but that a lesser stress (depletion of GSH, exposure to AA alone) is sufficient to trigger apoptosis.

Work from several laboratories has indicated that mitochondrial damage may represent a common early event in cell injury caused by toxic agents (66). Mitochondrial damage is initially manifested by a decrease in mitochondrial membrane potential ($\Delta\Psi_m$) followed by ATP depletion (67). In hepatocytes, death inducers, such as salicylate (68), glycochenodeoxycholate (69), 1,3 dichloropropanol (70), lipopolisaccharide (71), and ethanol (72), cause a $\Delta\Psi_m$ disruption that precedes signs of apoptosis or necrosis. Two major processes are likely candidates as mechanisms for a loss of $\Delta\Psi_m$: nonspecific damage to the inner mitochondrial membrane or a more specific process, the PT, due to the opening of the mitochondrial permeability pore (66). Mitochondrial membrane potential was assessed by flow cytometry after double staining with rhodamine 123 and PI. Exposure of E47 cells to BSO (59), AA (73), and Fe+AA (74) increased the percentage of cells that showed low rhodamine 123 fluorescence but were not stained with PI. This population refers to cells that are still viable but with damaged mitochondria, showing that these CYP2E1-dependent models of toxicity affect mitochondria before the onset of cell death (i.e., early event). This early mitochondrial damage was prevented by antioxidants, linking oxidative stress to mitochondrial damage. That mitochondria are an important target for CYP2E1-mediated oxidative stress is suggested by the fact that overexpression of mitochondrial catalase is capable of protecting cells that overexpress CYP2E1 against the toxicity induced by BSO (59), AA (64), and Fe+AA (60). If the decrease of mitochondrial membrane potential depends on the opening of the permeability transition pore, then a specific inhibitor should decrease the loss of mitochondrial membrane potential induced

by the toxic agents. Cyclosporin A inhibited the loss of $\Delta\Psi_m$ and the toxicity in CYP2E1-expressing cells exposed to AA (64), AA + Fe (61), and BSO (63), suggesting a role for the permeability transition on mitochondrial depolarization and subsequent toxicity. Additional evidence for increased mitochondrial damage in CYP2E1-overexpressing cells include the following: (*a*) Depletion of GSH by BSO treatment induced high lipid peroxidation of the mitochondrial fraction in E47 cells but not C34 cells, which was blocked by overexpression of catalase (59). Depletion of GSH decreased oxygen uptake in permeabilized cells with all respiratory substrates, and vitamin E prevented this decrease (54). (*b*) In CYP2E1-overexpressing cells treated with BSO + Fe-NTA, levels of ATP were lowered, and this was associated with a decreased rate of oxygen consumption by permeabilized cells with substrates donating electrons to complexes I, II, and IV of the respiratory chain. This mitochondrial damage was prevented by vitamin E (56). (*c*) ATP levels decreased rapidly after exposing AA-loaded E47 cells to Fe-NTA (57).

HepG2 cells were originally chosen for the establishment of CYP2E1-expressing cells because these cells expressed the auxiliary enzymes required for P450 function, e.g., NADPH-cytochrome P450 reductase and cytochrome b5; they are of human origin; and they maintain several classical liver functions, such as albumin synthesis, lipoprotein synthesis, and gluconeogenesis. Nevertheless, a limitation in their use is that they are a tranformed cell line. Studies were therefore performed to extend some of the key features observed for CYP2E1-dependent toxicity in HepG2 cells to nontransformed, intact hepatocytes. Treatment with pyrazole was used to increase the level of CYP2E1 (two- to threefold) and hepatocytes from pyrazole or saline-treated rats were placed into culture for 1–3 days and studied. Incubation with 100 mM ethanol (75) or 60 μM AA (75) resulted in loss of viability of hepatocytes from the pyrazole-treated rats, with lower effect on the hepatocytes from the saline-treated rats. Toxicity of AA and ethanol was reduced by exogenous addition of trolox (antioxidant) (75). Toxicity of AA was associated with increased lipid peroxidation and mitochondrial damage and was inhibited by retroviral overexpression of catalase (64). Therefore, toxicity features observed in CYP2E1-overexpressing HepG2 cells were reproduced in CYP2E1-overexpressing hepatocytes, suggesting that these are not unique characteristics of transformed cell lines.

Polyunsaturated fatty acids have been shown to be important in the development of alcoholic liver injury (76). There is growing evidence that only mildly increased levels of iron may cause toxicity to the liver in the presence of ethanol (77). We therefore considered the possibility as to whether there is a synergistic toxic effect of iron and AA (as a representative polyunsaturated fatty acid), especially in cells that express CYP2E1 (57). To test this hypothesis, the CYP2E1-expressing cells were exposed to Fe-NTA, AA, or a combination of iron and AA, and the effect on cell viability was compared with the effect in control HepG2 cells or HepG2 cells expressing CYP3A4 (the principal form of P450 in human liver). This cellular model was previously shown to be successful in demonstrating toxicity of Fe-NTA by itself (56) or AA itself (55) in HepG2 cells that overexpress CYP2E1. The toxicity of iron and AA was also evaluated in rat hepatocytes with high

levels of CYP2E1 (isolated from pyrazole-treated rats) and compared with controls (isolated from saline-treated rats). In this study, the working concentrations of Fe-NTA and AA were chosen such that the toxicity of these compounds by themselves was kept to a minimum. Thus, Fe-NTA (10 μM) alone or AA (5 μM) alone showed low toxicity to the CYP2E1 expressing HepG2 cells (E47 cells) (18% and 5%, respectively, at 24 h), whereas the combination produced synergistic injury (62% toxicity at 24 h). Exposure of cells not expressing any cytochrome P450 or HepG2-C3A4 cells (expressing CYP3A4) to 10 μM Fe-NTA plus 5 μM AA produced lower toxicity (14% and 32%, respectively), demonstrating a role for P450, and in particular CYP2E1, in the development of toxicity by exposure to Fe+AA. Lipid peroxidation was induced in the E47 cells exposed to Fe+AA and the synergistic toxicity was prevented by antioxidants, which also decreased lipid peroxidation. Similar results were obtained with other polyunsaturated fatty acids other than AA. Damage to mitochondria plays a role in the CYP2E1-dependent toxicity of Fe+AA because the mitochondrial transmembrane potential decreased early in the process and cyclosporin A prevented the toxicity. Toxicity in E47 cells exposed to Fe+AA is mainly necrotic in nature. Hepatocytes from pyrazole-treated rats, with high levels of CYP2E1, were more sensitive to Fe+AA toxicity than were saline control hepatocytes. Thus, results with the HepG2 cells plus Fe+AA could be replicated in normal hepatocytes. In summary, low concentrations of iron and AA that are not cytotoxic by themselves can act as priming or sensitizing factors for CYP2E1-dependent loss of viability in HepG2 cells or rat hepatocytes. This synergistic toxicity was associated with elevated lipid peroxidation and could be prevented by antioxidants, which prevent lipid peroxidation. Damage to mitochondria by CYP2E1-derived oxidants seems to be an early event in the overall pathway of cellular injury. Relatively low concentrations of iron or AA were effective in promoting toxicity in the CYP2E1-expressing cells, suggesting that interactions between CYP2E1, iron, and polyunsaturated fatty acids may lower the threshold concentrations for these reactive nutrients for inducing a state of oxidative stress, which may play a role in the development of alcohol-induced liver injury.

Although the experimental evidence provided points to a toxic role of ROS produced by CYP2E1 in a hepatic cellular model, recent evidence suggests that one consequence of the high rates of production of ROS by CYP2E1 is its own labilization and subsequent rapid degradation (78). This may be a regulatory mechanism to prevent high levels of the enzyme from accumulating within the cell. In E47 cells, steady state levels of CYP2E1 were increased by proteasome inhibitors and trolox (78). In another study (79), the activity of the CYP2E1 gene promoter was inhibited by oxidative stress (exogenous addition of H_2O_2 or overexpression of CYP2E1, effects blocked by catalase), suggesting an alternative site for the negative feedback of ROS on CYP2E1 expression. This phenomenon could be part of a concerted adaptive response to oxidative stress, consisting not only of repression of endogenous ROS-generating systems, but also induction of antioxidant defenses. In CYP2E1-expressing HepG2 cells (E47 cells) the alpha and microsomal GST mRNA were increased four- and twofold, respectively, together

with a twofold increase in enzymatic activity. Catalase mRNA and enzymatic activity were increased by twofold (80), and glutamate-cysteine ligase heavy or catalytic subunit (GCLC) and glutamate-cysteine ligase light or modulatory subunit (GCLM) mRNA and protein were increased by twofold with respect to control HepG2 cells (C34 cells) (53, 81). The increase in activity and expression of GCL, the rate-limiting enzyme of GSH synthesis, led to higher levels of GSH in CYP2E1-expressing cells than non-CYP2E1-expressing cells (53, 81). The increase in alpha and microsomal glutathione transferases and GCLC mRNA was caused by increased transcription. These activations in E47 cells were prevented by antioxidants, suggesting that ROS generated by CYP2E1 were responsible for the up-regulation of these antioxidant genes. GCLC and GCLM mRNA expression and protein levels were further increased when E47 cells were challenged with substrates for CYP2E1 or prooxidants, which further elevated oxidative stress, such as ethanol, AA, and Fe+AA (81). The increase in mRNA in treated E47 cells was blocked by antioxidants and by a CYP2E1 inhibitor. The transcriptional up-regulation mediated by CYP2E1-derived ROS seems to operate through a redox-sensitive element (ARE4) localized 3.1 kilobases upstream of the transcription start site in the GCLC gene (81). The mechanisms controlling the induction of genes by oxidative stress involve the activation of transcription factors, such as NF-$\kappa\beta$, AP-1, and Nrf2, which could mediate such inductions (82). A role for NF-$\kappa\beta$ in the protection of hepatocytes against CYP2E1-dependent oxidative stress has been recently suggested: Pyrazole-induced hepatocytes treated with AA for 24 h increased the translocation of NF-$\kappa\beta$ to the nucleus and increased NF-$\kappa\beta$ DNA binding activity (83). In addition, ARE4 contains an embedded AP-1 binding site; the role of these regulators in the induction of protective genes in E47 cells remains to be determined.

Designing strategies to counteract CYP2E1-mediated oxidative stress in hepatic cells requires a detailed understanding of the mechanisms involved. It is crucial to have knowledge of the role of intracellular triggers of stress, participation of the MPT and the contribution of apoptosis and necrosis. CYP2E1 inhibitors, such as diallylsulfide, phenethyl isothiocyanate, chlormethiazole, and polyenylphosphatidylcholine, have been used in vivo to prevent alcohol-induced oxidative stress and alcohol-induced liver toxicity (84–88). A variety of nonselective and selective caspase inhibitors are available that can inhibit initiator and/or executioner caspases. Inhibition of caspases inhibited apoptosis in CYP2E1-overexpressing cells treated with BSO (63), AA, and ethanol (77). Special attention must be taken for the possibility of apoptotic/necrotic switch of the mode of cell death. Cyclosporin A and ursodeoxycholate can inhibit the MPT (89). Antioxidants, such as tocopherol; GSH precursors, such as S-adenosylmethionine; and chemical mimics of antioxidant enzymes, such as metalloporphyrins, could represent possible therapies. Inducers of gene expression or gene therapy may be employed to boost expression of survival genes, such as heat shock proteins, or protective genes, such as superoxide dismutase and catalase. Many of these therapeutic pharmacological strategies are currently under basic investigation in our HepG2 cell model and in pyrazole hepatocytes.

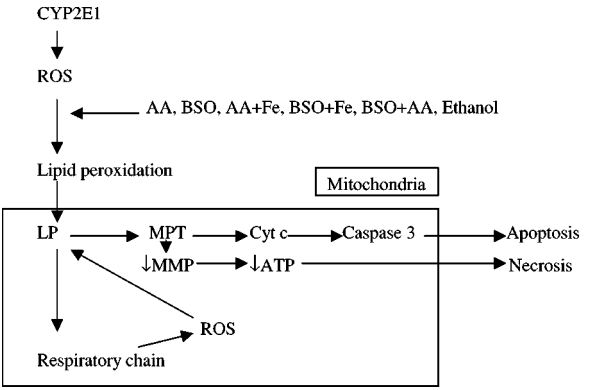

Figure 1 Proposed model for CYP2E1-dependent toxicity in hepatic cells.

Figure 1 presents a model that we believe explains many of the results obtained in the studies that were mentioned. CYP2E1-dependent ROS production results in enhanced lipid peroxidation when the hepatic cells are incubated with several inducers. The resulting peroxidation of biological membranes causes damage to the mitochondrial membrane. Damage to the mitochondria results in initiation of a membrane permeability transition, followed by a decrease in the mitochondrial membrane potential. The latter lowers cellular ATP levels, which promotes a necrotic type of cell death, whereas the former results in release of cytochrome c into the cytosol, which activates caspase 3, ultimately promoting an apoptotic type of cell death.

Figure 2 presents a scheme of the cellular adaptive response to CYP2E1-mediated oxidative stress. The protective cellular response may involve repression of endogenous ROS-generating system (by labilization of CYP2E1 protein or CYP2E1 transcription repression) and activation of antioxidant defenses, probably through the activation of transcription factors such as NF-$\kappa\beta$.

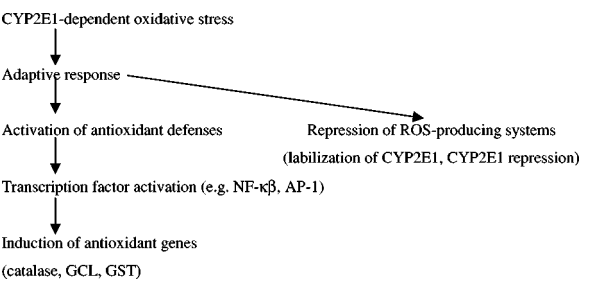

Figure 2 Adaptive responses to oxidative stress.

FUTURE PERSPECTIVES

With respect to alcohol-induced liver injury, such injury is likely to be a multifactorial process involving several mechanisms as reviewed in (4); the role of CYP2E1 in contributing to this injury requires further study. Indeed, some studies suggest that CYP2E1 may not play a role in alcohol liver injury [90, 91; reviewed and discussed in (4, 92)]. The regulation of CYP2E1 protein levels is complex, with transcriptional, translational, and posttranscriptional effects observed; some unifying concepts, e.g., how fasting and obesity each increase CYP2E1; how alcohol increases CYP2E1 protein, whereas alcohol-generated cytokines down-regulate CYP2E1 transcription, would be important to define. Most studies on the biochemical and pharmacological actions of CYP2E1 are derived from studies with rodents and rabbits and cultured hepatocytes; extrapolation to human studies will obviously be necessary. The role of polymorphic forms of CYP2E1 on CYP2E1 expression, activity, and action requires further understanding, as current literature suggests some possible relationships with certain types of cancers but not with alcohol toxicity. Are there endogenous substrates for CYP2E1? At present, acetone and some fatty acids (omega-1 hydroxylase activity) appear to be physiological substrates for CYP2E1, but further study should be carried out because altered metabolism of such putative endogenous substrates, if any, could contribute to the cellular actions associated with activated CYP2E1. CYP2E1 is present, although at relatively low levels, in other tissues, e.g., kidney, lung, brain, gastrointestinal tract. Much less is known about the actions of CYP2E1 under various pathophysiological conditions or after chronic ethanol exposure in these tissues. CYP2E1-nutritional interactions require further study, especially interactions with prooxidants, such as iron; polyunsaturated fatty acids; or reagents that lower antioxidant defense, e.g., lower GSH levels. There is much current interest in synergistic interactions between alcohol and hepatitis B or hepatitis C virus, especially with respect to generating oxidative stress. The role of CYP2E1 in such synergistic interactions, if any, would be important to explore in view of the many chemicals and conditions that are known to elevate CYP2E1.

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