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Forum Review

Induction and Regulation of Hepatocyte Apoptosis by Oxidative Stress

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ABSTRACT

Reactive oxygen intermediates (ROI) have been implicated in the induction of hepatocyte apoptosis that results from a variety of forms of liver injury. Exogenous oxidants induce hepatocyte apoptosis and may mediate death during inflammatory liver injury. Lethal levels of intracellularly generated ROI resulting from hepatotoxin metabolism, or the induction of enzymes in the cytochrome P450 family, are also important inducers of apoptosis. In addition, ROI production may mediate death from a number of diverse factors, including tumor necrosis factor-α, bile acids, ischemia, and transforming growth factor-β1. Oxidants alter many redox-sensitive cellular signaling pathways, including mitogen-activated protein kinases and transcription factors such as activator protein-1 and nuclear factor-κB. The mechanisms of oxidant-induced hepatocyte apoptosis remain unclear, but probably involve effects on cell signaling, as well as direct chemical interactions. The delineation of stimulus-specific mechanisms of oxidant-dependent hepatocyte apoptosis is important to the design of effective therapies for a number of forms of liver injury. *Antioxid. Redox Signal.* 4: 759–767.

INTRODUCTION

HEPATOCYTES are continuously exposed to variable levels of reactive oxygen intermediates (ROI) that are generated from multiple sources. Like any mammalian cell, hepatocytes constantly generate intracellular ROI as a by-product of normal mitochondrial respiration. However, the liver is unique in that it is the largest solid organ in the body and receives a large proportion of the body's blood supply. These two factors lead to a high level of hepatic oxygen consumption. Under normal physiological circumstances, a small proportion of this oxygen is converted to ROI in the form of superoxide ($O_2^{\cdot -}$), and hydrogen peroxide (H_2O_2), which in the presence of iron can be converted to hydroxyl radicals ($^{\cdot}OH$). In altered physiological states, such as liver growth, exposure to hepatotoxins, or inflammatory liver injury, hepatocytes are exposed to superphysiological levels of ROI. Hepatocytes have an elaborate system of enzymatic and nonenzymatic antioxidant defenses to remove or neutralize these ROI (26). However, excessive levels of ROI may overwhelm these hepatocellular antioxidant defenses, resulting in oxidative stress and hepatocyte injury and cell death. Death may take the form of necrosis or apoptosis depending on a number of factors, the most important being the level of ROI that the hepatocytes are exposed to. This review will discuss the inducers, cell-signaling pathways, and mechanisms of oxidant-induced hepatocyte apoptosis. A separate review in this forum will consider cell death from necrosis. However, it should be recognized that apoptosis and necrosis should no longer be considered separate and distinct entities (40). It is therefore somewhat artificial to separate the discussion of these two forms of cell death, because oxidant-induced apoptosis and necrosis share common initiators and mechanisms.

OXIDANT-DEPENDENT FORMS OF HEPATOCYTE APOPTOSIS

Exogenous ROI

Considerable evidence supports the fact that the direct exposure of hepatocytes to exogenous oxidants induces apoptotic cell death. Apoptosis can be induced by H_2O_2 or superoxide generated by the vitamin K analogue menadione in

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cultured hepatocytes and hepatoma cell lines (30, 32, 48). These findings are relevant to the mechanisms of hepatocyte cell death *in vivo*. During liver injury, activated Kupffer cells and recruited monocytes and neutrophils release ROI that may in part explain the ability of these inflammatory cells to promote liver injury (28). Death from exogenous oxidants is again not exclusively apoptotic, but can be necrotic as well (56, 67). Apoptosis may be converted to necrosis with higher concentrations of oxidants that induce changes in cellular metabolism or interact with and inactivate proteins, thereby blocking the energy- and protein-dependent process of apoptosis. Prominent among the effects of oxidants that have been implicated as mechanisms in the conversion of apoptosis to necrosis are cellular ATP depletion and the redox-mediated inactivation of caspases (56).

Intracellularly generated ROI

In addition to apoptosis resulting from direct extracellular oxidant exposure, a number of factors induce hepatocyte apoptosis by triggering the intracellular generation of ROI. Hepatotoxins in particular may trigger apoptosis through their direct metabolism to oxidants, or their induction of enzyme systems that generate ROI. Acute ethanol administration caused apoptosis in cultured hepatocytes and hepatoma cells (36), and chronic ethanol consumption led to hepatocyte apoptosis in rats and mice (5, 19). Ethanol induces ROI production and lipid peroxidation in hepatocytes and whole liver, and ethanol-induced death can be blocked by antioxidants (47). Apoptosis induced by a number of other hepatotoxins involves oxidant stress. Acetaminophen (APAP) is metabolized to a toxic metabolite that depletes cellular glutathione (GSH) and is associated with ROI generation leading to apoptosis (17). ROI have also been implicated as mediators of apoptosis from galactosamine (50) and microcystin (12). Anticancer drugs have been shown to induce apoptosis in hepatoma cells by both oxidant-dependent (25) and -independent pathways (41).

Critical to the metabolism of many compounds that results in hepatocyte apoptosis are the cytochrome P450 (CYP) enzymes. Both ethanol (66) and APAP (39) are metabolized by the CYP isoform CYP2E1, and this metabolism leads to induction of apoptosis from oxidative stress. In addition, CYP2E1 in the absence of substrate exhibits enhanced NADPH oxidase activity resulting in production of superoxide and H_2O_2 (13). CYP2E1 expression is increased in both alcoholic (63) and nonalcoholic steatohepatitis (64, 65), and the production of CYP2E1-generated ROI has been implicated as a mediator of hepatocyte injury and cell death in these two diseases (2, 37). However, the role of CYP2E1 in the pathogenesis of steatohepatitis remains controversial. Additional CYP isoforms besides CYP2E1 produce metabolites that generate ROI-dependent apoptosis, such as in CYP3A-mediated toxicity from the diterpenoid-containing herb skullcap (21).

ROI-dependent apoptosis from bile acids

The accumulation of bile acids within hepatocytes occurs in cholestatic liver diseases and may play a role in liver injury and cell death. Hydrophobic bile acids induce apoptosis in cultured hepatocytes and hepatoma cells (55, 70). Death occurs in association with ROI production and can be inhibited by antioxidants (70). Injury from bile acids also triggers the mitochondrial permeability transition (MPT), and death can be blocked by MPT inhibitors (70). Hydrophilic bile acids, such as ursodeoxycholic acid, not only do not induce apoptosis, but also can block apoptosis from hydrophobic bile acids, ethanol, Fas ligand, and transforming growth factor-β1 (TGF-β1) (54). Ursodeoxycholic acid inhibits ROI production and MPT induction by these agents (54). The differential death effects of hydrophobic and hydrophilic bile acids are related to their divergent effects on the phosphatidylinositol 3-kinase and nuclear factor-κB (NF-κB)-dependent cell signaling pathways (55). Activation of these two antiapoptotic pathways by hydrophilic bile acids may act to prevent the MPT and the ultimate generation of toxic ROI (55).

Death receptor-induced apoptosis

Tumor necrosis factor-α (TNF-α) plays an integral role in hepatocyte injury and cell death in a number of pathophysiological states, including liver injury from toxins, ischemia/reperfusion, and hepatitis virus. The signaling pathway of TNF-α-induced cell death has been carefully delineated and involves ligand binding to the type I TNF receptor, recruitment of a series of intracellular proteins, and the activation of caspases whose proteolytic activities mediate apoptotic cell death. However, multiple TNF-α death pathways exist in hepatocytes as cell death from caspase-independent apoptosis and necrosis has also been described (31, 42). Hepatocytes are inherently resistant to TNF-α toxicity, but become sensitized to TNF-α-induced death when the normal induction of TNF-α-dependent protective genes is blocked. Evidence exists that the process of sensitization may in part be oxidant mediated. TNF-α has been found to cause ROI generation in hepatocytes in some investigations (1, 45, 51), but not others (60, 69). In some studies, ROI generation only occurred at high, unphysiological TNF-α doses (1, 45), raising a question of the significance of these findings. However, detection of TNF-α-induced ROI may also be difficult because of their transient production in a localized cellular compartment, such as the mitochondria. TNF-α-dependent ROI induction occurs in whole liver mitochondria after partial hepatectomy (38), but the cell type responsible for ROI production was not determined in these studies, and may not have been hepatocytes. Mitochondria are a likely source of ROI resulting from TNF-α treatment. TNF-α suppresses mitochondrial respiration, and this effect is magnified during the sensitization of rat hepatocytes to TNF-α killing by coadministered cycloheximide (60). Mitochondrial dysfunction may lead to superoxide generation and trigger MPT, as will be discussed subsequently.

Considerable interest has centered on the role of antioxidants in regulating hepatocyte sensitization to TNF-α-induced apoptosis, because of the presumed role of oxidative stress in this killing. Initial investigations focused on the enzymatic antioxidants as inducible protective genes. Studies in nonhepatic cell types suggested that TNF-α-induced upregulation of the antioxidant enzyme manganese superoxide

dismutase (MnSOD) mediated TNF-α resistance. However, although TNF-α dramatically induced hepatocyte and whole liver MnSOD mRNA, no change in protein levels or enzyme activity occurred (10). Thus, basal MnSOD expression may play a role in the protection against TNF-α cytotoxicity, but MnSOD is not the critical TNF-α-induced factor that controls hepatocyte resistance from apoptotic death. No evidence suggests that any other antioxidant enzymes are up-regulated by TNF-α in hepatocytes, or regulate resistance from TNF-α-induced cell death. Therefore, studies have addressed the function of the principal nonenzymatic hepatocellular antioxidant GSH. TNF-α alone (1), or in combination with the sensitizing agent actinomycin D (45, 51), has been found to deplete primary hepatocytes (1, 45) or a TGF-α-overexpressing mouse hepatocyte line (51) of GSH. However, the interpretation of these data is complicated by the marked GSH fluxes that occur in primary hepatocytes following isolation and perfusion (3). No GSH depletion occurred with actinomycin D/TNF-α treatment of a nontransformed rat hepatocyte line (69). Additional evidence against a causal role for GSH depletion in TNF-α-induced apoptosis is the failure of GSH supplementation to inhibit cell death (69). Although the role of active GSH depletion in TNF-α toxicity remains unclear, it is evident that the preexistent GSH content of the hepatocyte significantly affects cell death. Although chemical depletion of GSH is insufficient by itself to sensitize hepatocytes to TNF-α killing, a reduction in cellular GSH content worsened cell death from actinomycin D/TNF-α (39). *In vivo* GSH depletion also increased mortality in a mouse model of TNF-α toxicity (39). Contradictory findings of a reduction in TNF-α toxicity from GSH depletion (23) can be explained as an artifact of the use of a protocol of acute, massive drops in GSH content that likely inactivated death pathway signaling. The fact that hepatocellular GSH depletion worsens TNF-α toxicity may be relevant to injury from this cytokine in alcoholic liver disease, which is marked by mitochondrial GSH depletion (16). Hepatocytes from ethanol-fed rats were in fact sensitized to TNF-α toxicity by a GSH-dependent mechanism, although death in this case was necrotic (8).

The involvement of ROI in the Fas/CD95 death receptor pathway has not been carefully examined in hepatocytes. Diet-induced GSH depletion has been shown to worsen Fas injury *in vivo* (22). However, ROI generation or GSH depletion has not been documented in Fas-induced cell death. In contrast, activation of the Fas death pathway can result from hepatocellular oxidative stress. In the HepG2 hepatoma line, death from chemotherapeutic agents was associated with p53-dependent increases in CD95 and Fas ligand (46). Induction of Fas ligand by bleomycin resulted from oxidative stress (25). Oxidant-induced Fas signaling triggered cell death because it could be prevented by inhibition of the CD95–Fas ligand interaction. Similarly copper-induced HepG2 cell death was dependent on oxidative stress and Fas ligand (62). A possible mechanism of oxidant-induced hepatocellular apoptosis is therefore through activation of the CD95 death receptor pathway. Interestingly, oxidant-dependent apoptosis from toxic bile acids results from ligand-independent CD95 oligomerization (15), although this process is likely upstream of oxidant generation.

TGF-β1-induced apoptosis

In addition to its role as a hepatocyte growth regulator, the cytokine TGF-β1 can also induce apoptotic cell death (52, 57). Treatment of hepatocytes or the hepatoma cell line Hep3B with TGF-β1 resulted in ROI generation and apoptotic cell death that could be blocked by antioxidants (33, 52, 57). TGF-β1 treatment of hepatocytes has also been reported to decrease antioxidant gene expression, which could potentially heighten oxidant-induced apoptosis. In rat hepatocytes, TGF-β1 decreased catalase mRNA levels (11, 33), and in some reports MnSOD and CuZnSOD levels as well (33). Other investigations have suggested transcriptional mechanisms for the regulation of TGF-β1-induced apoptosis (14, 52, 58), which will be discussed subsequently.

Apoptosis resulting from ischemia/reperfusion

In many clinical settings, prolonged ischemia followed by reperfusion results in liver injury and cell death. ROI are generated in the liver during reperfusion, although it remains unclear whether these are generated intracellularly in the hepatocyte or produced exogenously by Kupffer cells or neutrophils (27). The mechanisms of ischemia/reperfusion injury are complex, involving cytokines, inflammatory cell infiltrates, and ROI, and vary depending on whether the ischemia is cold or warm. However, ROI are thought to be critical for liver injury and cell death to occur. This injury results in primarily a sinusoidal endothelial cell death in cold ischemia (7), but hepatocyte and endothelial cell death following warm ischemia (18). Although several studies have found that death is apoptotic (9, 34), other investigations have documented an overwhelmingly necrotic death (20). This discrepancy may result from reliance on the nonspecific TUNEL assay in the studies concluding that death was from apoptosis, but this issue remains to be resolved.

INTRACELLULAR SIGNALING IN OXIDANT-DEPENDENT APOPTOSIS

Although ROI were formerly thought to affect cells strictly through their direct biochemical effects, it is now known that oxidants have profound influences on cell signaling cascades. Thus, in oxidant-induced apoptosis, death may not be the result of a lethal level of cellular damage occurring from processes such as lipid peroxidation. Rather activation or inhibition of cell signaling cascades may enter the cell into an apoptotic death pathway. An understanding of these ROI-induced changes in hepatocyte signaling cascades, and their involvement in the progression from injury to cell death, may suggest new therapies to block oxidant-induced apoptosis.

Mitogen-activated protein kinases

The mitogen-activated protein kinases (MAPK) are a family of serine/threonine kinases that include extracellular signal-regulated kinase (ERK), c-Jun NH_2-terminal kinase (JNK), and p38 MAPK. A variety of extracellular and intracellular factors trigger cytoplasmic kinase cascades that

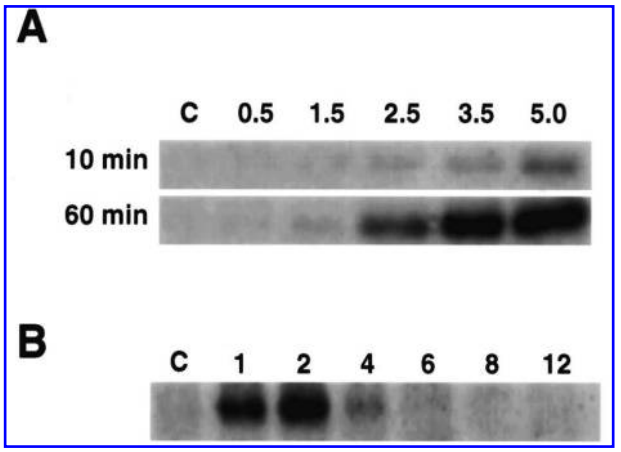

FIG. 1. H_2O_2 induces JNK activity in HuH-7 cells. Nuclear extracts were isolated from untreated HuH-7 cells (control; lane c) or cells treated with 0.5–5.0 m*M* H_2O_2 for 10 or 60 min (**A**), or with 2.5 m*M* H_2O_2 over a time course of the indicated number of hours (**B**). Proteins were incubated with the substrate glutathione *S*-transferase (GST)-c-Jun and [γ-^{32}P]ATP. Phosphorylated substrate was detected by immunoblotting. Higher, toxic concentrations of H_2O_2 were associated with increased JNK activity as shown. (Reprinted from reference 67 with permission of the American Physiological Society).

phosphorylate and activate MAPK. Activated MAPK then phosphorylate and activate transcription factors, including c-Jun, activating transcription factor-2 (ATF-2), and Elk-1. Oxidants are known activators of MAPK in nonhepatic cell types, but limited information exists on the effects of ROI on cultured hepatocytes or whole liver. In the human hepatoma cell line HuH-7, H_2O_2 induces JNK activation (Fig. 1). This results in increased activator protein-1 (AP-1) transcriptional activity because the AP-1 family member c-Jun is a substrate for JNK. AP-1 activation leads to cell necrosis as expression of an antisense c-Jun construct inhibited cell death (67). Hepatic JNK activation also occurred in mouse liver following oxidative injury from carbon tetrachloride (44). JNK activation was accompanied by decreased p38 MAPK activity (44), which was one of the first examples of independent regulation of these two MAPK. This divergence in JNK and p38 MAPK activation may reflect oxidant effects on phosphatases as well as kinases. Carbon tetrachloride-induced hepatic injury also resulted in increased mRNA expression of MAP kinase-phosphatase-1 (MKP-1), which down-regulates ERK and p38 MAPK. Little else is known about the function of ERK and p38 MAPK in oxidant-induced apoptosis in hepatocytes except that both mediate growth factor-induced inhibition of apoptosis from TGF-β1 (53).

Transcription factors

Transcriptional regulation of specific cellular genes is thought to play an important role in the cell's response to environmental stresses, such as oxidants. Transcriptional activation is frequently the end product of signaling cascades, such as the MAPK pathways (Fig. 2). Although many of these transcription factors are considered to be modulators of the cell's proliferative response, they may also regulate cell death. Investigations using the carbon tetrachloride model of oxidant-induced hepatic injury have demonstrated that a number of transcription factors are activated during injury from this compound. A prominent part of this response is the activation of the AP-1 family of genes. After acute carbon tetrachloride-induced injury, rapid increases in mRNA levels for *c-jun*, *junB*, *junD*, and *c-fos* have been demonstrated by *in situ* hybridization or northern blotting (24, 44, 59). Increased AP-1 DNA binding resulted with c-Jun, JunB, JunD, c-Fos, and ATF-2 all present in the activated complex (44). By immunoblotting only JunD, protein levels were increased in the 4 h after injury, suggesting that the other AP-1 subunits were activated by the phosphorylation of preexisting proteins. As discussed previously, elevated JNK activity preceded the increase in AP-1 DNA binding and presumably played a role in the activation of AP-1 subunits. JunD expression was localized to the pericentral hepatocytes (44), which undergo cell death from carbon tetrachloride. Although it was suggested

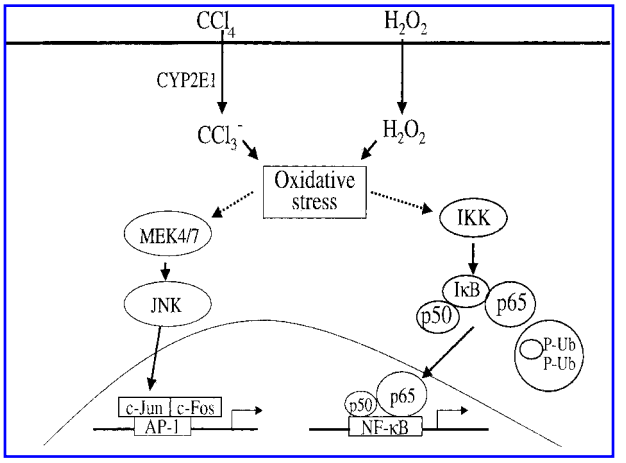

FIG. 2. Oxidant-induced signaling cascades cause transcriptional activation in hepatocytes. An oxidative stress is created in the hepatocyte from H_2O_2 exogenously produced by inflammatory cells, or hepatotoxin metabolism such as that from the conversion of carbon tetrachloride (CCl_4) by the cytochrome P450 isoform 2E1 (CYP2E1) to the trichloromethyl (CCl_3^-) free radical. The resultant oxidant stress created in the cell triggers two signaling cascades, the JNK/AP-1 and NF-κB pathways, that result in transcriptional activation. JNK activation typically results from its phosphorylation by the upstream kinases MEK4/7. Activated JNK phosphorylates c-Jun, increasing its transcriptional activity. c-Jun heterodimerizes with other AP-1 family members, such as c-Fos, and binds to the AP-1 site of specific genes, thereby causing transcriptional activation. The classical activation pathway for NF-κB is through phosphorylation of IκB by IκB kinase (IKK). This phosphorylation triggers IκB ubiquitination and subsequent degradation by the 26S proteosome. IκB degradation unmasks NF-κB's nuclear translocation signals, allowing it to translocate to the nucleus and stimulate the transcription of target genes. How hepatocellular oxidative stress activates the JNK and NF-κB pathways remains to be determined as indicated. However, it has been demonstrated that both AP-1- and NF-κB-dependent gene products modulate hepatocyte death from oxidative stress.

that JunD function was to block cell proliferation (44), JunD may in fact regulate cell death.

In vitro studies indicate that activation of the AP-1 family member c-Jun may be a critical event that promotes hepatocyte apoptosis from oxidative stress. As already discussed, the induction of AP-1 activation in HuH-7 cells by H_2O_2 led to necrosis, because death was prevented by inhibition of c-Jun function (67). Adenoviral mediated inhibition of c-Jun function also inhibits H_2O_2- and menadione-induced apoptosis in rat hepatocytes (M.J. Czaja, unpublished data). Activation of the JNK/c-Jun/AP-1 pathway may therefore be a critical event in oxidant-induced hepatocyte cell death. However, the mechanism by which AP-1 promotes cell death is unknown.

Another transcription factor activated by stress or injury, including that from ROI, is NF-κB. Both H_2O_2 and menadione cause NF-κB activation in rat hepatocytes (Fig. 3). Hepatic NF-κB activation also occurred during carbon tetrachloride-induced oxidative stress (43). Inhibition of NF-κB activation *in vitro* decreased hepatocyte death from both H_2O_2 and menadione (30), suggesting that NF-κB activity was critical for apoptosis to occur. This finding contrasts with the known protective effects of NF-κB activation in the TNF-α (6, 68) and bile acid (55) death pathways. Hepatocyte apoptosis can therefore be either promoted or inhibited by NF-κB depending on the nature of the apoptotic stimulus. The mechanisms of these divergent NF-κB activities remain to be delineated, but these findings underscore the fact that the mechanisms regulating hepatocellular apoptosis can vary greatly with different initiating factors.

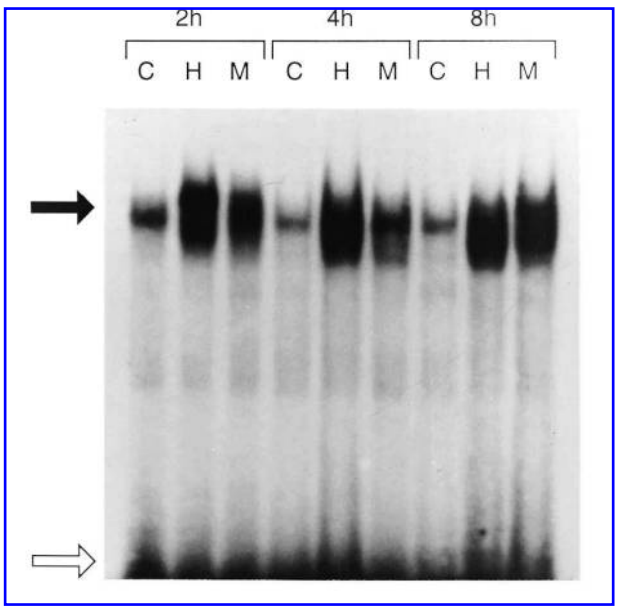

FIG. 3. H_2O_2 and menadione induce NF-κB activation by electrophoretic mobility shift assay. Nuclear protein was isolated at the indicated times from untreated control RALA255–10G hepatocytes (C), or cells treated with H_2O_2 (H) or menadione (M). Aliquots were used for electrophoretic mobility shift assays with an NF-κB consensus oligonucleotide. The autoradiogram demonstrates increased NF-κB DNA binding (solid arrow) after treatment with either H_2O_2 or menadione. Open arrow indicates free probe. (Reprinted from reference 30 with the permission of the American Physiological Society).

Besides the direct effects of exogenous oxidants on transcriptional activity, intracellularly generated ROI may also induce apoptosis by transcriptional mechanisms. One such example is TGF-β1-induced apoptosis. As previously discussed, TGF-β1-induced apoptosis is associated with ROI generation and impairment of antioxidant gene expression, and death is prevented by antioxidants (14, 57). ROI generation and GSH depletion in fetal hepatocytes are blocked by inhibition of protein synthesis with cycloheximide (58), suggesting that gene expression is essential for oxidative stress and death to occur. In the Hep3B hepatoma cell line, expression of the TGF-β1-inducible transcription factor TIEG1 caused oxidant-dependent apoptosis, suggesting that this factor may mediate cell death from TGF-β1 (52). Other investigations in the HuH-7 hepatoma cell line, demonstrated that TGF-β1 inhibited expression and phosphorylation of the retinoblastoma gene product (14). Retinoblastoma gene expression blocked TGF-β1-induced apoptosis, and the mechanism in part was through regulation of the transcription factor E2F-1 (14). Further investigations are needed to integrate these two pathways and determine whether these transcriptional regulators modulate TGF-β1-induced apoptosis in nontransformed hepatocytes.

MECHANISMS OF OXIDANT-INDUCED APOPTOSIS

Overwhelming oxidative stress

Several general mechanisms have been proposed to mediate oxidant-induced hepatocyte apoptosis. The simplest possible mechanism is that apoptosis is triggered by overwhelming oxidant-induced chemical damage to cellular macromolecules. When cellular oxidant levels exceed the capacity of antioxidant defenses, oxidative stress results and ROI may react with and damage lipids, proteins, and DNA. The hepatocyte may somehow sense when a critical level of cellular injury has occurred, and activate an apoptotic cell death pathway. In support of this possibility is the fact that antioxidants are critical in hepatocellular protection against cell death. Inhibition of the enzymes that inactivate H_2O_2, catalase and glutathione peroxidase, sensitized cultured rat hepatocytes to death from H_2O_2 (61). Glutathione peroxidase knockout mice were similarly sensitized to hepatic cell death from neutrophil generated ROI produced during liver injury from galactosamine and lipopolysaccharide (29). The generation of oxidative stress may be further modulated by the cellular levels of factors other than antioxidants, such as hepatocyte iron, which increases the conversion of H_2O_2 to the more toxic hydroxyl radical (49). Inhibition of apoptosis from APAP (17) and bile acids (70) by antioxidant supplementation can be taken both as evidence that apoptosis is oxidant-mediated, and that death occurs from direct cellular injury by ROI. However, antioxidants may in fact affect cell survival by altering redox-sensitive pro- or antiapoptotic signaling cascades that regulate death. Inhibition of cell death may occur from these effects on cell signaling and be unrelated to direct cellular injury from processes such as lipid peroxidation, which are in fact secondary effects.

Death by cell signaling

If redox changes in the cell initiate cell death by affecting signaling pathways, then ROI would be acting as upstream initiators of apoptosis, rather than downstream effector molecules. As discussed previously, in bleomycin-induced apoptosis in HepG2 cells, cell death was mediated by CD95–Fas ligand interaction (25, 46). Antioxidants prevented GSH depletion and reduced induction of Fas ligand (25), suggesting that bleomycin-induced ROI caused apoptosis by induction of the Fas-death receptor pathway rather than through direct cellular damage. In ischemia/reperfusion liver injury, ROI have been shown to mediate transcriptional activation. Adenoviral expression of the antioxidant enzyme MnSOD in mice significantly decreased hepatic ischemia/reperfusion injury (71). MnSOD overexpression also significantly reduced AP-1 and NF-κB activation (71), indicating that ROI generation was an upstream inducer of these factors, rather than a downstream consequence of their activation. Although this investigation did not prove that AP-1 or NF-κB directly mediated liver injury in this model, the results suggest the possibility that these transcription factors, and not the direct chemical effects of ROI, may mediate hepatocyte death in ischemia/reperfusion injury. In support of this possibility are the findings that AP-1 and NF-κB promote oxidant-induced death in liver cells *in vitro* (30, 67). As hepatocyte redox-sensitive signaling pathways are further delineated, additional investigations will have to determine the relationship between ROI generation and signal activation, as well as the role of specific signals in cell death.

Role of mitochondria

A common feature of either chemical or signal-mediated mechanisms of oxidant-induced apoptosis may be the critical involvement of mitochondria (35). Numerous studies have shown that various forms of apoptosis occur as a result of the MPT. The MPT is a rapid increase in the permeability of the outer and inner mitochondrial membranes resulting in collapse of the electrochemical gradient, mitochondrial swelling, and release into the cytosol of proapoptotic mitochondrial factors such as cytochrome *c* (35). A number of forms of oxidant-dependent hepatocyte apoptosis occur through this mitochondrial pathway, including that caused by TNF-α (6), bile acids (70), APAP (17), and TGF-β1 (54). A number of factors initiate the MPT, including ROI. ROI may therefore serve as second messengers in death pathways through their induction of MPT. Alternatively, MPT initiation may be ROI-independent, but one of the consequences of the MPT is the generation of mitochondrial ROI. Mitochondrial generated ROI may then directly damage cells or activate proapoptotic signals.

PREVENTION OF OXIDANT-INDUCED APOPTOSIS

The realization that oxidant-induced apoptosis contributes to hepatocyte cell death in a number of forms of liver injury raises new therapeutic possibilities for these disorders. Therapeutic interventions can be aimed at oxidant neutralization, altering proapoptotic oxidant-induced signaling cascades, or blocking common effectors of apoptosis such as caspases. Knowledge of the mechanisms of cell death will allow more targeted, and hence possibly more effective, therapies. Instead of employing global antioxidant therapy, single antioxidant genes can be specifically targeted to the organelle responsible for ROI generation, such as the delivery of MnSOD to block ischemia/reperfusion injury (71). The complexities of the effects of changes in redox homeostasis on cell function complicate the general applications of such therapies. For example, mitochondrial expression of an antioxidant gene inhibited death from ischemia/reperfusion (71), but promoted apoptosis from TNF-α (4). Low levels of oxidants may in some instances be critical for maintaining cytoprotective and regenerative cell functions, whereas high levels initiate apoptosis. It is therefore necessary to delineate further the pathways of oxidant-induced hepatocellular apoptosis in order to develop therapies targeted specifically at events related to cell death.

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ABBREVIATIONS

AP-1, activator protein-1; APAP, acetaminophen; ATF-2, activating transcription factor-2; CYP, cytochrome P450; ERK, extracellular signal-regulated kinase; GSH, glutathione; H_2O_2, hydrogen peroxide; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; MnSOD, manganese superoxide dismutase; MPT, mitochondrial permeability transition; NF-κB, nuclear factor-κB; ROI, reactive oxygen intermediates; TGF-β1, transforming growth factor-β1; TNF-α, tumor necrosis factor-α.

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