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Mitochondrial function in liver disease

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1. ABSTRACT

Oxidative stress is involved in the pathogenesis and progression of different liver diseases, such as alcoholic liver disease and biliary cirrhosis. The increased mitochondrial production of O_2^- at complexes I and III, and consequently of H_2O_2 and other reactive oxygen species (ROS), triggered by NADH overproduction seems the major cause of mitochondrial and cellular oxidative stress and damage in chronic alcoholism. The mitochondrial oxidative stress renders hepatocytes susceptible to ethanol- or acetaldehyde-induced mitochondrial membrane permeability transition (MMPT) and apoptosis. Nitrosative stress contributes to cell death by peroxynitrite formation. The expression of the death receptor ligand CD95 is also up-regulated by acetaldehyde metabolism. Consequently, a dual mechanism, NADH-driven MMPT and CD95-mediated apoptosis, involving in both cases acetaldehyde metabolism and ROS production, operates in ethanol-induced apoptosis. In the biliary cirrhosis induced by chronic cholestasis, liver mitochondria show increased H_2O_2 production and GSH depletion and oxidation. Dysfunctional hepatocytes, with a loss in mitochondrial cardiolipin and decreased mitochondrial membrane potential evolve during cholestasis to apoptosis. Ursodeoxycholic acid prevents enlargement of this population as well as mitochondrial oxidative stress. Mitochondrial oxidative stress precedes the initiation and execution of hepatocyte apoptosis in chronic alcoholism and biliary cirrhosis. We suggest that overproduction of mitochondrial NADH is the primary cause for the development of alcoholic and non-alcoholic liver disease by a situation of chronic mitochondrial oxidative stress, which should be considered the second hit that renders hepatocytes susceptible to cell injury and apoptosis.

2. MITOCHONDRIA AND CELL SIGNALING IN LIVER PATHOPHYSIOLOGY

Mitochondria play a key role in the initiation and progression of liver diseases as the mitochondrial primary products superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) and other down-stream reactive oxygen species (ROS) up-regulate the expression of pro-inflammatory cytokines and trigger death signals. Mitochondria are also essential in the execution of hepatocyte apoptosis through both the intrinsic and Bid-dependent extrinsic pathways. In addition, mitochondrial oxidative stress contributes to oxidative damage and necrotic cell death.

Mitochondrial impairment plays an important role in liver diseases through the ROS-induced release of reactive lipid peroxidation products, such as 4-hydroxynonenal and malondialdehyde, and cytokines, such as TNF-alpha and Fas ligand that trigger mitochondrial permeability transition and apoptosis (1). 4-hydroxynonenal contributes to fibrogenesis by enhancing the production of TGF-beta by macrophages and collagen by activated stellate cells (2-4). Consequently, the mitochondrial products of lipid peroxidation and cytokines become involved in alcoholic and non-alcoholic liver diseases, particularly steatohepatitis (1,5).

On the other hand, mitochondrial and cellular damage may lead to the formation of anti-mitochondrial antibodies. Primary biliary cirrhosis (PBC) is characterized by antimitochondrial antibodies, particularly by serum antibodies against pyruvate dehydrogenase complex E2 subunit (PDC-E2)(6). Although almost all cells in the body have mitochondria, the presence of these autoantibodies is associated with specific destruction of biliary cells (7). The reason for this specific destruction is still unknown. Several mechanisms appear to be involved in the formation of antimitochondrial antibodies. Cross-reactivity with mycobacterial hsp65 may be one of such mechanisms. Thus, IgG3 antibodies that cross-react with the major mitochondrial autoepitope and a motif of *Lactobacillus delbrueckii* have been recently found (6). Environmental factors have also been proposed as causative agents in PBC since the lipoyl domain of PDC-E2 might be replaced by a chemical xenobiotic mimic that breaks self tolerance (8). This is the case of 2-octynoic acid, a cosmetic and food additive widely used in perfumes, lipstick and food flavorings, which seems to modify PDC-E2 leading to formation of autoantibodies in PBC patients (8).

The role of mitochondrial defects in severe liver disease can not be overlooked; for instance, mitochondrial fatty acid oxidation disorders lead to hypoketotic, hypoglycemia, metabolic acidosis and hepatic failure in newborns, causing sudden unexpected deaths (9).

This review focuses on the key role of mitochondrial oxidative stress in the pathophysiology of alcoholic liver disease and biliary cirrhosis. A common mechanism for alcoholic and non-alcoholic liver diseases is proposed.

3. MITOCHONDRIAL OXIDATIVE AND NITROSATIVE STRESS IN LIVER DURING CHRONIC ALCOHOLISM

Chronic alcoholism in animals and humans leads to oxidative stress in the liver, evidenced by increased hepatic lipid peroxidation (10-15). Oxidative stress is particularly intense in liver mitochondria from alcoholic rats as indicated by glutathione depletion and by the increases in glutathione oxidation, GSSG release into the bile and lipid peroxidation (10, 16-17). Furthermore, chronic ethanol consumption leads to a much greater increase in the content of carbonyl groups in mitochondrial proteins than in cytosolic proteins (18). Mitochondrial NADH overproduction seems to be the major responsible for this oxidative stress as explained below. The referred evidence (10-18), that includes the use of organ non-invasive techniques (10-11), solved an early (1980-1990) dispute in the literature regarding the occurrence of liver lipid peroxidation in alcoholism.

Acetaldehyde metabolism occurs mainly in liver mitochondria and leads to mitochondrial ROS generation through NADH formation. Indeed, the H_2O_2 production that occurs in isolated liver mitochondria incubated with acetaldehyde is prevented by inhibition of aldehyde dehydrogenase with disulfiram (16). We propose that the increased H_2O_2 and ROS production triggered by acetaldehyde in chronic alcoholism is the major cause for glutathione oxidation in liver mitochondria and that in turn, the increase in GSSG levels contribute to the rise in ROS production driven by substrates of the mitochondrial respiratory chain.

Several years ago we found that incubation of isolated hepatocytes with ethanol or acetaldehyde leads to GSH depletion (19). Now, we have found that chronic administration of ethanol to rats causes GSH depletion and an increase in GSSG levels in liver mitochondria (16). Consequently, the GSSG/GSH ratio increases as a sign of oxidative stress in hepatic mitochondria during chronic alcoholism. This oxidative stress is aggravated with time during chronic alcoholism since glutathione oxidation was more intense when the administration of ethanol continued for several months (Figure 1A).

The physiological generation of O_2^- and H_2O_2 at complexes I and III (20) was found increased and associated with mitochondrial glutathione oxidation during chronic alcoholism (16, 21-22). Accordingly, the rate of mitochondrial H_2O_2 production increased progressively during chronic alcoholism (Figure 1B). Furthermore, administration of S-adenosyl methionine (SAM) to alcoholic rats restored mitochondrial GSH

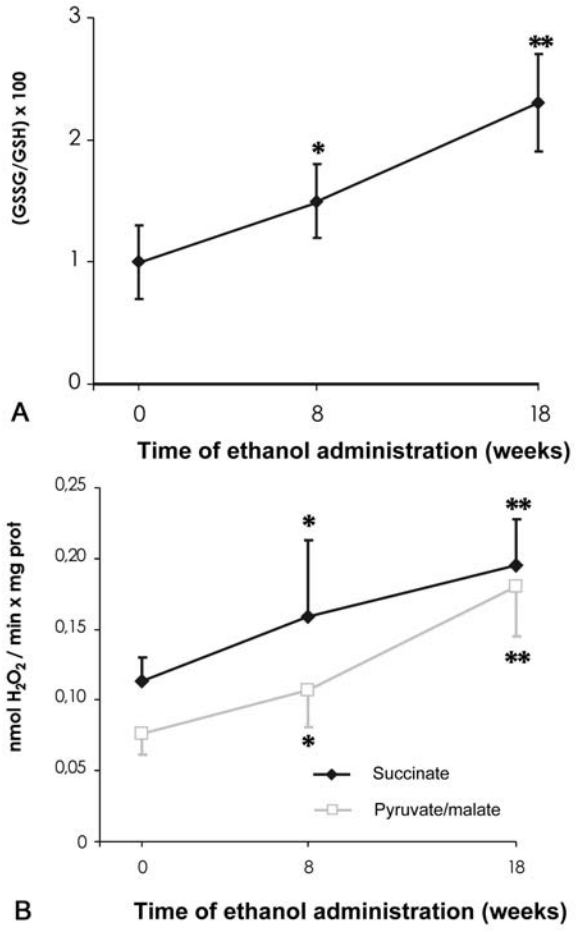

Figure 1. A. Effect of chronic ethanol feeding on oxidized to reduced glutathione ratio (GSSG/GSH) in rat liver mitochondria. B. Effect of chronic ethanol administration on the rate of H_2O_2 production in liver mitochondria added with 5 mM pyruvate-2.5 mM malate (complex I-linked substrate) or 10 mM succinate (complex II-linked substrate). Rats were pair-fed with the Lieber-De Carli liquid diet containing 36% of calories as ethanol or with an isocaloric mixture with maltose instead of ethanol (16). The amount of ethanol consumed daily by the ethanol-fed group was approximately 12 g of ethanol/kg of body weight. The study was approved by the Research Committee of the University of Valencia School of Medicine (Valencia, Spain). The rate of H_2O_2 production in liver mitochondria was determined as described (16). The number of experiments was 4-8. Statistical difference is indicated as follows: * $P < 0.05$; ** $P < 0.01$ versus the control group.

levels, the glutathione redox ratio and diminished mitochondrial H_2O_2 production (Figure 2).

It remains to be elucidated which is first, if mitochondrial glutathione oxidation or the increased O_2^- and H_2O_2 production in liver mitochondria. Murphy and co-workers reported that oxidation of mitochondrial glutathione induces an increase in mitochondrial ROS formation through glutathionylation of complex I (23). Therefore, glutathione oxidation may contribute to the increase in mitochondrial ROS generation associated with chronic alcoholism. Furthermore, a vicious circle is likely to occur due to a positive feed back between glutathione oxidation and O_2^- and H_2O_2 generation in mitochondria.

On the other hand, the rise in mitochondrial O_2^- and H_2O_2 formation may also be ascribed to alterations in the mitochondrial complexes that are sources of O_2^- and H_2O_2 by chronic ethanol exposure (22). Indeed, chronic alcoholism is associated with decreased levels of the iron-sulfur centers in complex I, which causes higher levels of FMN semiquinone and consequently, higher rates of O_2^- production (22,24). A decrease in cytochrome b caused by chronic ethanol feeding would also lead to increased levels of ubisemiquinone and to higher rates of O_2^- production (22,25-26). Moreover, a marked decrease in the heme content of cytochrome c oxidase induced by chronic alcoholism (25) may enhance mitochondrial O_2^- production. It is noteworthy that all these changes are associated with a decrease in the activity of all the mitochondrial respiratory complexes, except complex II, following chronic ethanol exposure (27). Furthermore, the steady-state levels and the synthesis rate of all 13 polypeptides encoded by the mitochondrial genome diminished following chronic ethanol consumption due to alterations in the function of mitochondrial ribosomes (26,28). Repeated ethanol exposure also causes accumulation of un-repaired mitochondrial DNA lesions leading to depletion of hepatic mitochondrial DNA, which may account for this reduced polypeptide synthesis (29-30).

The increased mitochondrial O_2^- generation may contribute to nitrosative stress when O_2^- reacts with NO to give $ONOO^-$ (peroxinitrite). Indeed, protein nitration, which is a marker of peroxinitrite formation, increases in the liver upon chronic ethanol consumption (31-32). Protein nitration also rises after incubation with acetaldehyde in hepatocytes from alcoholic rats (16). Mitochondrial NOS would be the major source of NO to form peroxinitrite in mitochondria (33). On the other hand, peroxinitrite may inhibit mitochondrial complexes and this blockade of electron transfer would contribute to a higher mitochondrial O_2^- generation.

4. MITOCHONDRIAL OXIDATIVE STRESS IN BILIARY CIRRHOSIS. RELEVANCE FOR THERAPY

4.1. Chronic cholestasis: a model of free radical disease

Cholestasis occurs in numerous chronic human diseases including primary biliary cirrhosis, primary sclerosing cholangitis, allograft rejection, iatrogenic obstruction of bile ducts and biliary atresia (34). In cholestasis, elevated bile acid concentrations within the liver promote liver injury, liver cirrhosis and failure. Toxic bile acids induce hepatocyte injury by apoptosis (35), necrosis (36), and eventually, biliary fibrosis and cirrhosis (37).

Oxidative stress is involved in the development of cholestatic liver injury. Thus, hydrophobic bile acids

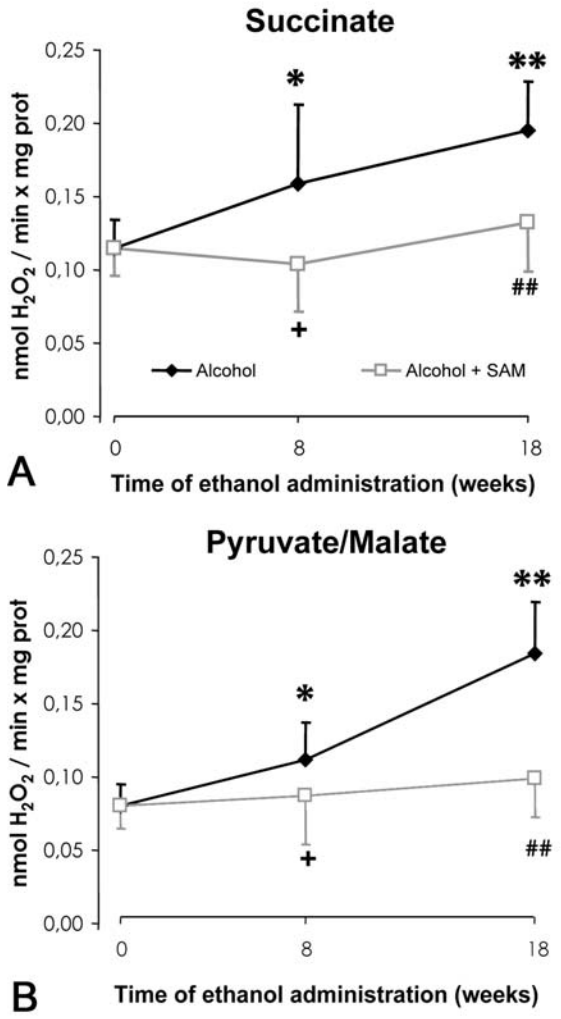

Figure 2. Effect of S-adenosyl methionine (SAM) administration on the rate of H_2O_2 production in liver mitochondria from alcoholic rats. The number of experiments was 3-6; SAM was given subcutaneously at a daily dose of 12 mg/kg body weight. Exptl. conditions as in Figure 1. Statistical difference is indicated as follows: * $P < 0.05$, ** $P < 0.01$ versus the control group at time 0; # $P < 0.05$; ## $P < 0.01$ versus the alcohol group.

stimulate the generation of ROS in hepatocytes (38-39), and in liver mitochondria (38,40). In addition, oxidative injury has been reported in rats receiving hydrophobic bile acids intravenously (41) and in the bile duct-ligated model of chronic cholestasis in rats (34, 42).

Experimental evidence supports the concept that bile salt cytotoxicity induces mitochondrial dysfunction (43-44). Indeed, induction of mitochondrial membrane permeability transition (MMPT) by hydrophobic bile salts can be a critical step in hepatocyte death by either necrosis or apoptosis (36). *In vitro* studies have shown that toxic bile salts such as glycochenodeoxycholic (GCDC) induce MMPT in a dose-dependent manner (45). In contrast ursodeoxycholic acid (UDCA), a protective hydrophilic bile acid used in the treatment of primary biliary cirrhosis, inhibits the GCDC-induced MMPT and prevents hepatocyte necrosis. Consequently, a mechanism of UDCA cytoprotection may relate to inhibition of bile salt-induced MMPT. Deoxycholic acid, another hydrophobic bile acid, induces loss of the mitochondrial membrane potential (delta-Psi_m), increased ROS production and promoted hepatocyte apoptosis (40). Co-administration of UDCA with deoxycholic acid inhibits hepatocyte apoptosis both *in vivo* and *in vitro* (39). Interestingly, UDCA was also able to prevent hepatocyte apoptosis caused by non-membrane damaging agents such as ethanol, transforming growth factor-beta1 (TGF-beta1), anti-Fas antibody or okadaic acid (39). Even though the mechanism of the UDCA inhibition of apoptosis and of MMPT induction is lacking, there is a proposal that UDCA effect is due to binding to the pro-apoptotic protein Bax and by preventing Bax translocation from the cytosol to mitochondria where the initiation of the critical pathways of apoptosis occurs (40,46).

Recently, Sokol *et al* (41) hypothesized that hydrophobic bile acids accumulate intracellularly during cholestasis and interfere with normal mitochondrial electron transfer, inhibiting the activity of respiratory complexes and reducing ATP synthesis. In a recent study we focused on the rate of mitochondrial H_2O_2 generation in chronic cholestasis (47) and found that in state 4, using pyruvate/malate as NADH-linked substrates or succinate as $FADH_2$-linked substrate, liver mitochondria from cirrhotic rats generated three-fold more H_2O_2 than controls animals. The increased generation of O_2^- and H_2O_2 leads to mitochondrial oxidative stress as evidenced by mitochondrial glutathione oxidation. In addition, down-regulation of glutamate cysteine ligase expression led to a limitation in the capacity of GSH synthesis and consequently to a progressive reduction in the antioxidant capacity of the liver (47). Then, mitochondrial oxidative stress and oxidative damage occur in the hepatocytes with high levels of oxidative protein damage.

Inhibition of MMPT-dependent apoptosis can be regulated by mitochondrial cardiolipin content. In fact, cardiolipin provides an increased pool of negative ions that non-specifically bind Ca^{2+} and prevent binding to protein sites that induce opening of mitochondrial pores (48). Accordingly, an increase in mitochondrial cardiolipin content was observed by Lieser *et al* (49) in the early phase of cholestasis as a protective mechanism. However, we reported that this adaptation is lost later in the course of chronic cholestasis and cardiolipin content decreased after three weeks of cholestasis (47). According to Paradies at al, mitochondrial ROS production may cause oxidative cardiolipin damage which leads to cardiolipin depletion and impairment of respiratory chain activity (50).

On the other hand, we found that UDCA administration in chronic cholestasis increases antioxidant defence by up-regulating the expression of

gamma-glutamate cysteine ligase and consequently increasing GSH synthesis, prevents the mitochondrial cardiolipin loss and reduces mitochondrial ROS production (47). Taken together, these effects correlate with a low level of hepatocyte apoptosis and of liver oxidative damage.

4.2. Uncoupling protein (UCP) as an antioxidant agent and new therapeutic target: friend or enemy?

Mitochondrial ATP synthesis depends on the coupling of sequential oxido-reductions occurring in the respiratory chain with ADP phosphorylation. The primary process of electron transfer is closely associated with generation of O_2^- and H_2O_2, with the intramitochondrial dismutation catalysed by mitochondrial superoxide dismutase (Mn-SOD) (20).

The membrane-associated mitochondrial proteins termed uncoupling proteins (UCPs) act as proton transporters, dissipating the mitochondrial proton gradient and increasing thermogenesis while reducing the efficiency of ATP synthesis (51). They play important roles in protection from oxidative stress (52) and in regulating apoptosis (53-54). UCPs by uncoupling oxidative phosphorylation provide an additional advantage by constraining mitochondrial O_2^- and H_2O_2 production (55). Since the loss of ATP synthesis occurs early in liver disease, many compensating systems are quickly activated to maintain energy supply, *i.e.* increasing respiratory chain activity, cytochrome synthesis and mitochondrial biogenesis (56). If these mechanisms fail and ATP synthesis is not rapidly restored, the respiratory chain is overloaded and this paradoxically may increase O_2^- and H_2O_2 formation. In fact, the state 4 conditions with a high reduction level of the respiratory chain, combined with a limited rate of utilization of the proton electrochemical gradient in an O_2-rich environment, promote O_2^- formation.

Interestingly, recent studies have linked the activation of the UCP_2 protein in rat liver mitochondria to the suppression of H_2O_2 production (52,57). Taniguchi *et al.* (58) have reported an enhanced UCP_2 expression in biliary epithelial cells during primary biliary cirrhosis. They suggested that biliary epithelial cells in primary biliary cirrhosis are struggling against several stresses, oxidative included, even if the liver injury is mild. Thus, epithelial cells actively express UCP_2 which might be one of the essential capabilities for cytoprotection from oxidative stress. However, this could result in further ATP depletion, ultimately causing cell death (58). The molecular mechanisms that are involved in this UCP induction are unknown, but some evidences support the TNF-inducible origin of UCP_2. In summary, the induction of UCP_2 conveys some adaptive advantage during early phases of cholestasis. However, the up-regulation of UCP_2 may become deleterious in stressed mitochondria, i.e. upon GSH depletion or accumulation of toxic bile acids. Consequently, the mechanisms geared up by liver cells to prevent ROS accumulation may, in some circumstances and in the long term, worsen the oxidative stress situation and compromise cellular viability.

5. MITOCHONDRIAL OXIDATIVE AND NITROSATIVE STRESS TRIGGERS A DUAL MECHANISM FOR HEPATOCYTE APOPTOSIS.

Apoptosis may be the hidden companion of necrosis in the development of liver disease. Increased plasma aminotransferase activities and hepatic inflammatory infiltrate are hallmarks of necrosis, but apoptosis may be going on *in vivo* in hepatocytes without leaving apparent traces in plasma. Nevertheless, apoptosis seems to play an important role in the progression of alcoholic liver disease to cirrhosis (59). Thus, cirrhotic livers exhibit numerous apoptotic bodies (60) and chronic ethanol intake in animals increases apoptosis in liver (61-62).

Apoptosis may occur through the extrinsic pathway mediated by death membrane receptors which lead to formation of the death initiating signalling complex (DISC), or through the intrinsic pathway mediated by mitochondrial oxidative stress, dysfunction and membrane permeabilization that leads to apoptosome formation. Hepatocytes are considered as type II cells that require the mitochondria to proceed by the intrinsic pathway for apoptosis. Indeed, the caspase 8 activity released from DISC is low in hepatocytes and amplification of the apoptotic cascade through Bid-dependent mitochondrial release of Smac is needed to activate caspase 3 (63-64).

Chronic ethanol consumption depletes mitochondrial antioxidant levels leading to an increased susceptibility to alcoholic injury induced by apoptotic stimuli (65). Thus, mitochondria from chronic ethanol-fed rats are more sensitive to MMPT induced by different apoptotic stimuli, such as ceramide, GD3 ganglioside or recombinant Bax (66). Ethanol-induced defects in mitochondria seem to lead to an increased O_2^- and H_2O_2 formation that promotes apoptotic and necrotic cell death in response to otherwise benign challenges and contribute to the development of alcohol-induced liver disease (67).

A dual mechanism is involved in hepatocyte apoptosis during chronic alcoholism (16,68). NADH overproduction triggered by ethanol and acetaldehyde metabolism leads to mitochondrial oxidative stress which causes cell death through the intrinsic pathway. In addition, oxidative stress up-regulates Fas ligand (CD95L) expression which promotes cell death through the extrinsic pathway by autocrine or paracrine mechanisms.

Ethanol or acetaldehyde-induced apoptosis depends completely on mitochondria and requires opening of the mitochondrial membrane permeability transition (MMPT) pores since it is prevented by cyclosporine A (16). Mitochondrial oxidative and nitrosative stress play a critical role to trigger

acetaldehyde-induced MMPT as this cell death is prevented by antioxidant vitamins C and E or by L-NAME, which inhibits nitric oxide synthase (16). Prevention of oxidation of mitochondrial glutathione by administration of S-adenosyl methionine renders hepatocytes resistant to ethanol-induced apoptosis. Furthermore, overexpression of mitochondrial Mn-SOD blocked mitochondrial glutathione depletion and hepatocyte apoptosis in rats chronically fed with ethanol (69). Consequently, it seems that the susceptibility of hepatocytes from alcoholic rats to ethanol-induced apoptosis is due, at least in part, to modifications induced by glutathione oxidation in mitochondrial proteins, such as glutathionylation of the respiratory complexes.

A population of apoptotic hepatocytes evolves during biliary cirrhosis (47). These hepatocytes show a marked decrease in mitochondrial cardiolipin and in mitochondrial membrane potential. Again this cell death is associated with mitochondrial glutathione oxidation and a remarkable increase in mitochondrial H_2O_2 generation (47). Furthermore, administration of UDCA prevents at the same time mitochondrial oxidative stress and the decrease in cardiolipin and membrane potential abrogating the development of apoptosis. Therefore, mitochondrial H_2O_2 generation and glutathione oxidation are key modulators of hepatocyte apoptosis *in vivo* during biliary liver disease. Moreover, mitochondria also act at the initial stages for both the intrinsic and extrinsic pathways of apoptosis in several forms of liver disease.

6. IS OVERPRODUCTION OF MITOCHONDRIAL NADH A COMMON PATHOGENIC MECHANISM FOR ALCOHOLIC AND NON-ALCOHOLIC LIVER DISEASE?

Non-alcoholic steatohepatitis (NASH) and fatty liver are specially relevant in western countries because they are associated with obesity, diabetes and hypertriglyceridemia, being insulin resistance their common feature (70). Liver mitochondria from patients with NASH are swollen and exhibit paracristalline inclusions, loss of cristae, depletion of mitochondrial DNA and decreased activity of all respiratory chain complexes (71,72).

In patients with NASH, there is both an increased delivery of electrons to the respiratory chain from NADH and a restricted electron transfer rate, a combination that leads to increased levels of reduction in respiratory complexes I and III and to increased mitochondrial production of O_2^- and H_2O_2 (5,20). This may trigger several vicious circles by oxidizing mitochondrial DNA (mtDNA), proteins and lipids, by enhancing TNF-alpha formation and by depleting antioxidants; all of them would further block electron flow and increase mitochondrial O_2^- and H_2O_2 formation (5).

Consequently, mitochondrial O_2^- and H_2O_2 generation is increased in mice with fatty livers induced either by genetic obesity or methionine/choline-deficient diets (73). Furthermore, cumulative damage to mtDNA and a progressive decrease in the repair of mtDNA occur as fatty liver disease evolves. Diehl and co-workers (74) have proposed that genetic or acquired differences in DNA repair enzyme activities – particularly in mitochondria- might explain the inter-individual differences in the occurrence or outcome of this liver disease.

Day and James (75) proposed that the development of NASH involves two pathophysiologic hits: first peripheral insulin resistance which would increase lypolisis and delivery of free fatty acids to the liver leading to steatosis; and second some additional source of oxidative stress capable of initiating enough lipid peroxidation to overcome the cellular defense mechanisms and produce necroinflammation. Peroxidation of mitochondrial phospholipids may cause cell necrosis and the products of lipid peroxidation, particularly 4-hydroxynonenal and malondialdehyde may activate hepatic stellate cells, the major collagen-producing cells in the liver, stimulate leukocyte chemotaxis and activate nuclear factor kappaB contributing to inflammation (2,3,75). Recently, Sanyal *et al* (71) suggested that the second hit would involve a specific intrahepatic defect, most likely in the mitochondria, that would render the hepatocytes more susceptible to the oxidative stress triggered by increased fatty acid beta-oxidation promoting inflammation, cell death and fibrosis.

An increased mitochondrial beta-oxidation may limit lipid accumulation in the fat liver (5) . Thus, a rise in fatty acid oxidation occurs in genetically obese mice and in patients with NASH (71,76). Accordingly, serum beta-hydroxybutyrate levels, an index of fatty acid beta-oxidation, were higher in those patients with NASH than in controls and those with fatty liver (71) . We reported that beta-hydroxybutyrate mimicks the effect of acetaldehyde causing apoptosis only in hepatocytes from alcoholic rats but not in control rats (16). Ethanol and acetaldehyde oxidative metabolism generates excessive NADH during chronic alcoholism. Consequently NADH overproduction may be the common trigger of hepatocyte apoptosis and tissue damage in both alcoholic liver disease and NASH (Figure 3).

Excessive NADH generation is associated with increased mitochondrial O_2^- and H_2O_2 production and causes hepatocyte apoptosis only in those hepatocytes susceptible to MMPT. We propose that a chronic mitochondrial oxidative stress characterized by glutathione oxidation and chronic NADH overproduction would render hepatocytes especially sensitive to the apoptotic signal given by increased mitochondrial H_2O_2. Mitochondrial glutathione oxidation would trigger glutathionylation of mitochondrial complexes, oxidation of critical cysteins,

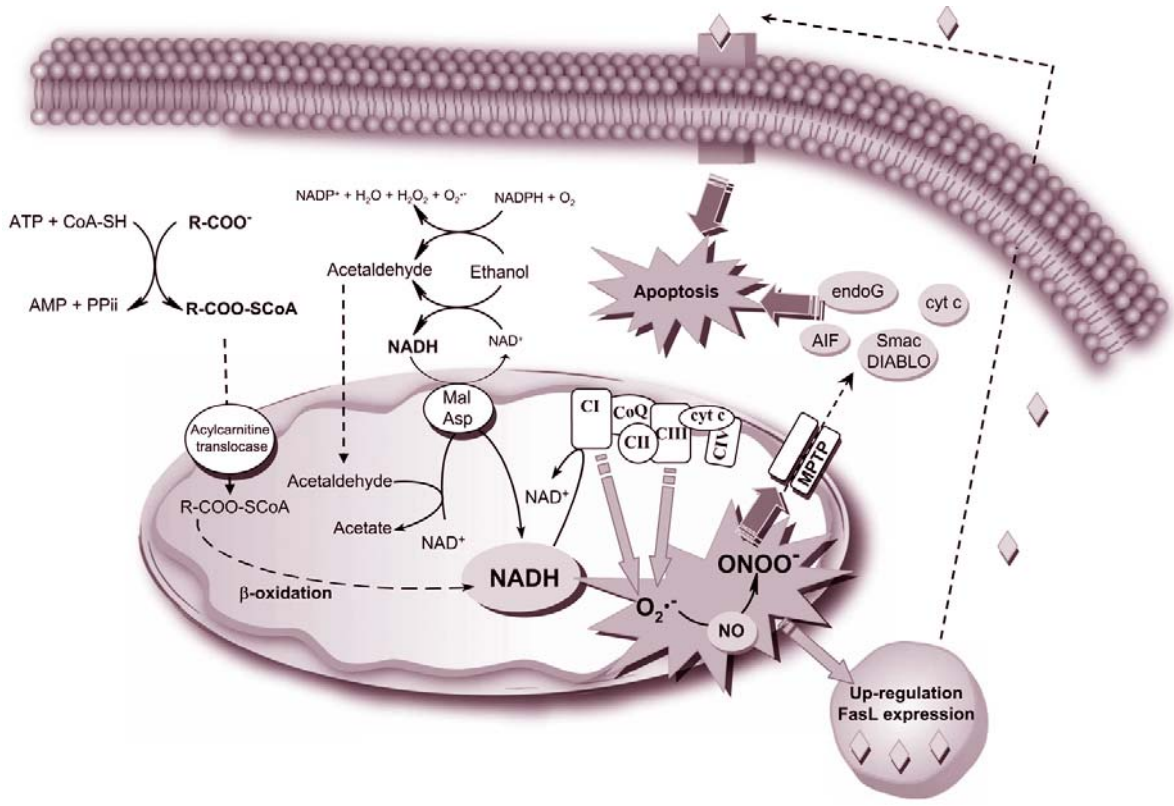

Figure 3. A common pathogenic mechanism involved in alcoholic and non-alcoholic steatohepatitis: NADH-driven mitochondrial O_2^- and H_2O_2 production and cell death.

nitration of mitochondrial complexes and lipid peroxidation and induction of O_2^- production. Consequently, chronic mitochondrial oxidative stress would be the second hit for development of NASH or alcoholic liver disease. A common pathophysiological mechanism for these liver diseases could be established since in fact, the histological findings for alcoholic and non-alcoholic steatohepatitis are very similar (74).

In favour of our hypothesis, the susceptibility of hepatocytes to cell death was abrogated when mitochondrial oxidative stress was prevented or markedly diminished either by administration of SAM in the case of chronic alcoholism, or by treatment with UDCA in the case of biliary cirrhosis (47). Thus, SAM treatment prevented glutathione oxidation in liver mitochondria from alcoholic rats (see Figure 2 and ref. 77) and acetaldehyde-induced cell death was markedly diminished in hepatocytes from alcoholic rats treated with SAM (16). On the other hand, UDCA prevented to a great extent mitochondrial glutathione oxidation and abrogated the presence of apoptotic hepatocytes during biliary cirrhosis (47).

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