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REVIEW

Ethanol-induced oxidative stress: basic knowledge

Mario Comporti · Cinzia Signorini · Silvia Leoncini · Concetta Gardi · Lucia Ciccoli · Anna Giardini · Daniela Vecchio · Beatrice Arezzini

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Abstract After a general introduction, the main pathways of ethanol metabolism (alcohol dehydrogenase, catalase, coupling of catalase with NADPH oxidase and microsomal ethanol-oxidizing system) are shortly reviewed. The cytochrome P_{450} isoform (CYP2E1) specifically involved in ethanol oxidation is discussed. The acetaldehyde metabolism and the shift of the NAD/NADH ratio in the cellular environment (reductive stress) are stressed. The toxic effects of acetaldehyde are mentioned. The ethanol-induced oxidative stress: the increased MDA formation by incubated liver preparations, the absorption of conjugated dienes in mitochondrial and microsomal lipids and the decrease in the most unsaturated fatty acids in liver cell membranes are discussed. The formation of carbon-centered (1-hydroxyethyl) and oxygen-centered (hydroxyl) radicals during the metabolism of ethanol is considered: the generation of hydroxyethyl radicals, which occurs likely during the process of univalent reduction of dioxygen, is highlighted and is carried out by ferric cytochrome P_{450} oxy-complex (P_{450}–$Fe^{3+}O_2^{\cdot-}$) formed during the reduction of heme-oxygen. The ethanol-induced lipid peroxidation has been evaluated, and it has been shown that plasma F_2-isoprostanes are increased in ethanol toxicity.

Keywords CYP2E1 isoform · Ethanol metabolism · Hydroxyethyl radicals · Liver-free non-protein bound iron · Oxidative stress · Plasma isoprostanes

M. Comporti (✉) · C. Signorini · S. Leoncini · C. Gardi · L. Ciccoli · A. Giardini · D. Vecchio · B. Arezzini
Department of Pathophysiology,
Experimental Medicine and Public Health,
University of Siena, via A. Moro, 53100 Siena, Italy
e-mail: comporti@unisi.it

Introduction

Ethanol unlike many other hepatotoxic chemicals is not a foreign substance for living organisms; it occurs, in fact, in small amounts in mammalian tissues [82] and is conceivably formed by the alcohol dehydrogenase-catalyzed reduction of acetaldehyde derived from the decarboxylation of the intermediary metabolite pyruvate [83]. A similar reaction also occurs in biological fermentation (such as that yielding ethyl alcohol in wine and other alcoholic beverages) in which glucose is fermented by yeast. Moreover, significant amounts of ethanol are normally formed in the gastrointestinal tract, absorbed by the portal vein and metabolized in the liver [64]; most alcohol is of microbial origin, the other portion probably arising from the acetaldehyde formed by the normal pathways of degradation of threonine, deoxyribose phosphate and β-alanine. Furthermore, ethanol is rapidly and most entirely converted to the key intermediate, acetate, which can enter a wide-spread variety of metabolic pathways. This "more physiological" aspect of ethanol when compared with other hepatotoxic drugs complicates to a large extent the study of the mechanisms involved in the pathogenetic effects of alcohol. The great interest on ethylism from both a clinical and a sociological point of views resulted in an impressive number of studies on the effects of ethyl alcohol. However, the results have not been even univocal because the models used (alcohol dosage, acute or chronic administration, animals studied, different susceptibility to ethanol consumption, sex, nutritional status, etc.) have been extremely variable. Even the choice of the model itself for the study is questionable: in fact, if the acute (single large dose of alcohol) intoxication is a model more suitable for the study of the direct effects of ethanol at the cellular, subcellular and molecular level, it is obviously away from the

conditions of the alcoholic; the chronic intoxication, on the other hand, is more adherent to such conditions but is subjected to a number of variables (of nutritional order in particular) that can contribute to a great extent to the development of the lesions, so that it is more difficult to discern the net role played by alcohol. Since the aim of the present review is to focus on the mechanisms of ethanol-induced liver injury and on the role of oxidative stress, particular attention will be paid to the results obtained with acute ethanol intoxication.

Ethanol metabolism and consequent alterations of the overall metabolism of the hepatic cell

The pathophysiological bases of the hepatic alterations produced by ethanol start obviously from its metabolism. Ethanol is quickly absorbed in the gastrointestinal tract and is almost completely oxidized to carbon dioxide and water. Minor amounts only are excreted, unmetabolized, in urine, breath and sweat. The rate of alcohol metabolism varies between 50 and 180 mg/h/kg body wt [118] in adult men, while in rats and mice, notwithstanding the natural aversion to alcohol, a two to threefold higher rate has been reported [67].

Alcohol is mainly metabolized in the liver, and the metabolic pathways are summarized in Fig. 1. The most important pathway is represented by alcohol dehydrogenase, a zinc containing metalloenzyme [121] widely distributed in nature, localized in the soluble cytoplasm and NAD^+-dependent. It catalyzes the oxidation of ethanol to acetaldehyde (Fig. 1A). It is generally accepted that ethanol is also metabolized by catalase, a heme-containing enzyme widely distributed in nature and particularly expressed in the peroxisomes. Catalase, which normally catalyzes the decomposition of H_2O_2 to H_2O and O_2 (catalatic reaction), can also catalyze the reduction of H_2O_2 to H_2O if electron donors are present (peroxidatic reaction); generally such reaction is catalyzed by peroxidase, but catalase can also catalyze peroxidative type reactions, when hydrogen peroxide is produced and a source of electron donors is available. Ethanol, which acts as an electron donor, is so oxidized to acetaldehyde (Fig. 1B). Hydrogen peroxide necessary for the reaction can be produced by oxidases, some of which also occur in the peroxisomes [14].

Ethanol oxidation can also result from the coupling of the reactions catalyzed by NADPH oxidase (microsomal) and xanthine oxidase with catalase (Fig. 1C, D, respectively). Which is the real importance of catalase in ethanol metabolism is not clear; it seems to be the most important pathway for the metabolism of methanol [1], while it would be of minor importance for the oxidation of ethanol, at least under normal conditions.

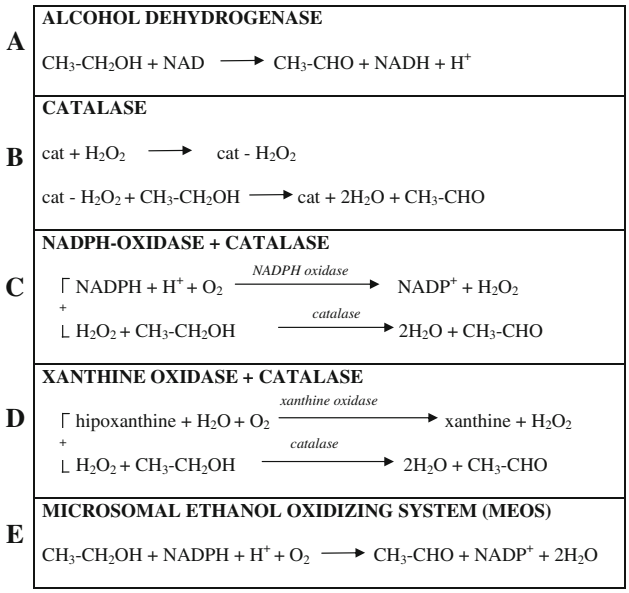

Fig. 1 Metabolic pathways of ethanol

Finally, Lieber and De Carli [69] and Lieber et al. [78] have described a third metabolic pathway for ethanol oxidation occurring in the endoplasmic reticulum of the hepatocyte and named "microsomal ethanol-oxidizing system" (MEOS) (Fig. 1E). It utilizes the terms of the enzymatic system of "drug metabolism" (mixed function oxidase system, driven by the microsomal electron transport chain). MEOS with NADPH as a cofactor, oxidizes, it too, ethanol to acetaldehyde (Fig. 1E). A long debate followed the report of MEOS as a separate and individual ethanol-oxidizing system: some authors [19, 115] claimed that microsomal ethanol oxidation could result from the coupling of the activities of NADPH oxidase, the real microsomal enzyme, with catalase or with catalase and alcohol dehydrogenase (present as contaminant in the microsomal fraction) [57]. However, the group of Lieber [117] and others [87] showed that (a) MEOS is active even in acatalasemic animals; (b) a reconstituted system consisting of the essential terms of drug metabolism, i.e., cytochrome P_{450}, NADPH-cytochrome *c* reductase and synthetic phospholipids, is able to oxidize, besides benzphetamine (characteristic substrate of drug metabolism), ethanol and other alcohols too and that MEOS is adaptively increased after chronic ethanol consumption, like other drug metabolizing activities. The induction of drug metabolizing enzymes due to alcohol [56, 73, 108, 109] and the fact that ethanol in vitro inhibits in a competitive way the same enzymes, could explain, at least in part, the increased tolerance of alcoholics to sedatives when sober and the enhanced sensitivity to sedatives when inebriated. The quantitative contribution of microsomal ethanol oxidation to the overall ethanol metabolism is another much

debated question. According to some estimations [115] this route does not play a significant role in the metabolism of alcohol in vivo; however, according to the calculation of [71] about ¼ of the ethanol ingested could be metabolized by MEOS in normal conditions, while, after prolonged ethanol consumption, the role of MEOS could be considerably greater. Today, it is well known that the isoform of cytochrome P_{450} involved in ethanol oxidation, CYP2E1 (see in the following paragraphs), has a much higher K_m for ethanol (8–10 mmol/l) compared to that of alcohol dehydrogenase (0.2–2 mmol/l); however, chronic alcohol consumption increases the activity of CYP2E1 by several times [3].

Acetaldehyde metabolism

As reported in the previous paragraphs, all the ethanol-metabolizing pathways lead to the formation of acetaldehyde. The latter can be oxidized to acetate mainly by enzymes, aldehydes dehydrogenases, which are mitochondrial, NAD^+-dependent enzymes. Thus, the two main steps of ethanol metabolism, namely, the NAD^+-linked oxidation of ethanol to acetaldehyde and the NAD^+-linked oxidation of acetaldehyde to acetate, cause an increased concentration of the reduced pyridine coenzyme, NADH. Therefore, a decrease in the NAD^+/NADH ratio occurs in the liver cell and such an imbalance would represent, according to a classic view of the problem [68, 74, 75], the central event in the explanation of the various metabolic alterations produced directly in the hepatic cell by ethanol oxidation, including triglyceride accumulation (the importance of the reductive stress environment created by ethanol oxidation will be outlined in the following paragraphs).

As is known, the reoxidation of NADH formed in the soluble cytoplasm occurs through shuttle mechanisms that transfer reducing equivalents from cytoplasmic NADH to mitochondrial electron transport chain. The latter becomes quickly saturated by reducing equivalents originating from ethanol and thus can slow down the citric acid cycle that, under normal conditions, supplies itself reducing equivalents to the respiratory chain. The slackening of the citric acid cycle leads to various metabolic consequences, the major part of which derive from decreased oxidation of acetate, which can originate, as mentioned in the previous paragraphs, directly by ethanol, even if a consistent part of the latter would be metabolized in peripheral tissues [80]. The metabolic unbalance derived from the decreased acetate oxidation is primarily reflected on fatty acid oxidation, fatty acid synthesis, ketone bodies formation and cholesterol metabolism. Decreased fatty acid oxidation and increased fatty acid synthesis have been demonstrated since a long time ago [40, 74, 95, 99, 126]. It was therefore suggested that ethanol, acting itself as a fuel for mitochondria, could replace in a "competitive way" fatty acids that, under normal conditions, represent the major source of energy for the liver cell.

Today, it is known that the ethanol-induced stimulation of hepatic triglyceride synthesis depends, to a great extent, upon the increased expression of the enzymes involved in fatty acid synthesis that are regulated by the transcription factor sterol regulatory element-binding protein (SREBP)-1 [123], located in the endoplasmic reticulum. Upon ethanol feeding, SREBP-1 is proteolytically cleaved to the active form that translocates to the nucleus, inducing the expression of the genes coding for lipogenic enzymes.

Pathogenetic mechanisms of the hepatic lesions produced by ethanol

As it is known, the long lasted debate whether nutritional deficiencies associated to alcoholism are responsible of liver injury [15, 49] or whether ethanol per se (or some proximal metabolite) exerts toxic effects on hepatic cell has been largely resolved by the introduction of a novel model for experimental studies based on liquid diets in which ethanol (36% of total calories) replaces in a isocaloric way part of carbohydrates. With these diets, considered nutritionally adequate, the alcohol assumption in the rat was greatly increased and hepatic lesions (hepatic steatosis) were obtained in both rats and humans [76, 77]. These lesions therefore apparently were independent of nutritional deficiencies. Subsequently, with the use of primates as experimental animals, a sequential production of fatty liver, inflammatory reactions, necrosis, fibrosis and sclerosis has been obtained [78] upon prolonged ethanol feeding.

The ethanol-induced ultrastructural changes of the liver cell involve both the mitochondria and the endoplasmic reticulum. The mitochondrial changes consist of enlargement, swelling, shortening and disorganization of the *cristae*, decreased number or absence of matrix granules and intramitochondrial crystalline inclusions. The presence of bizarre shapes and *giant mitochondria* [61, 63] with an increased matrix density was also reported [56, 66, 72, 97]. The changes involving the endoplasmic reticulum [109] consist of a marked proliferation of the smooth membranes with abundant vesicular structures. The alterations of the endoplasmic reticulum are, according to [56], the first morphological changes detectable in the liver cell after ethanol feeding to rats; they were observed, in fact, after few days of alcohol administration, while mitochondrial changes appeared some days later. Autophagic vacuoles, containing altered mitochondria, and residual dense bodies were also described. The presence of hyaline Mallory bodies was repeatedly documented. An increased number of peroxisomes were also reported [12], which is consistent with the increased catalase activity.

The morphological changes of mitochondria have a functional counterpart, consisting in a decrease in cytochrome *a*, a_3 and *b* [21, 62, 110], of the enzymatic activities of the Krebs cycle [21, 110] and in general of the respiratory control [45, 59]. Many of these alterations are stable and can be in turn responsible for the changes in lipid metabolism produced by ethanol oxidation itself. Ethanol-induced mitochondrial changes also involve the autochthonous synthesis of some mitochondrial proteins, thus producing an unbalance between proteins synthesized in mitochondria and those synthesized in the endoplasmic reticulum. Such unbalance could influence the mitochondria biosynthesis.

The toxic effects of acetaldehyde

That ethanol-induced liver changes are in part mediated by the proximal metabolite acetaldehyde that has been proposed since a long time ago and reconsidered when it has been observed [21] that many of the earlier mentioned ethanol-induced mitochondrial changes are reproduced by acetaldehyde. In particular, the addition of acetaldehyde to isolated mitochondria at concentrations of the same order as those occurring in the liver actively oxidizing ethanol (1–3 mM), inhibits mitochondrial respiration at the level of complex I (NADH-ubiquinone oxido-reductase) and the coupling site I of oxidative phosphorylation [21, 22]. Moreover, acetaldehyde inhibits, at the same concentrations, fatty acid oxidation, and such inhibition seems to be due to the inhibition of β-oxidation, citric acid cycle and oxidative phosphorylation [24]. The inhibition of the oxidation of NAD^+-dependent substrates [21] and the inhibition of fatty acid oxidation [24] do not seem to depend from a competition of acetaldehyde with such substrates for NAD^+. It has been suggested [23, 25], on the other hand, that acetaldehyde could interact with sulphydryl groups involved in oxidative phosphorylation particularly with those essential for the complex I (NAD^+ ubiquinone-oxydoreductase) which is the site of the respiratory chain mainly affected by both acetaldehyde and ethanol. Thus, the block of –SH groups would be responsible for the alterations. The reaction of acetaldehyde and other alkanals with cysteine has been actually known since many decades (formation of thiazolidine-carboxylic acids) and other low molecular weight thiols (reduced glutathione, GSH, in particular) can also be involved in this reaction. In effect, ethanol toxicity is always accompanied by a decrease in hepatic GSH content. If, as we will see later, lipid peroxidation is going to develop in ethanol hepatotoxicity, much more reactive aldehydes (alkenals and 4-hydroxyalkenals) will be formed and a much higher reactivity toward –SH groups and other nucleophiles has to be expected.

Finally, the acetaldehyde-produced alterations of the mitochondrial functions impair the mitochondrial acetaldehyde metabolism [51], thus producing worsening of the damage by a vicious cycle.

Ethanol-induced oxidative stress

It has been known since a long time ago that prior administration for a variety of substances known as antioxidants affords a marked protection against the liver damage induced by CCl_4 and other hepatotoxins [44, 54]. It was subsequently demonstrated [29, 31, 36] that CCl_4 both administered in vivo or added in vitro to liver preparations markedly increases the peroxidation of liver lipids, as measured by malonil dialdehyde (MDA) formation. Such a pro-oxidant effect of CCl_4 was immediately confirmed by Recknagel and Ghoshal [100, 101]. The theoretical background for these studies was suggested by [18] and [125], who proposed that in the hepatic cell, CCl_4 could undergo a homolytic cleavage yielding free radicals ($CCl_3^{\cdot}$, $CCl_3OO^{\cdot}$, etc.). The latter could rapidly interact with neighboring molecules, such as proteins, nucleic acid, thiols and membrane unsaturated fatty acids. The latter interaction would set into motion lipid peroxidation that seriously affects membrane structure and function. Since antioxidant pretreatment [37] was also found to be effective in ethanol-induced liver damage, it was suggested [38] that the liver injury produced by CCl_4 or ethanol could have a common pathogenetic mechanism, namely, oxidative stress[1] and peroxidation of liver lipids. It was subsequently found [30, 32] that, as in the case of CCl_4, the MDA production by incubated liver homogenates is greatly enhanced during 1, 2, 4, 6 and 12 h after acute ethanol administration. The increase in liver lipid peroxidation precedes the accumulation of triglycerides in the liver, thus excluding that the enhanced MDA formation could result from the increased hepatic fat content; the latter possibility is also ruled out by the fact that no increased MDA production occurs 24 h after ethanol dosing when the hepatic triglyceride level is maximum. Furthermore, it was shown [32] that ethanol added in vitro to liver homogenates has a pro-oxidant effect although to a lesser extent than carbon tetrachloride. The effect is specific for the liver tissue, since ethanol, either in vitro or in vivo, has no effect on the peroxidation of brain [48] or other tissue homogenates [114].

It was proposed [32] that ethanol or its metabolites stress the "peroxidative balance" of the liver cell [16] toward autoxidation, either acting as pro-oxidant or lowering the cellular antioxidant level. Direct evidence for increased

[1] Oxidative stress is generally considered as a disturbance in the pro-oxidant/antioxidant balance in favor of the former, leading to potential damage [47].

hepatic lipoperoxidation in vivo after acute ethanol intoxication was forwarded by Kalish and Di Luzio [58], who showed that the peroxide content was increased in the liver in ethanol-treated rats. Hashimoto and Recknagel [50], on the contrary, found no evidence of conjugated diene absorption characteristic of peroxidized lipids [17] in the lipids of any subcellular fraction at any time after acute ethanol intoxication. On the basis of these results, it was concluded that in the case of ethanol-induced liver injury, there is no direct evidence for the in vivo occurrence of hepatic lipoperoxidation. Di Luzio [39] questioned the above results and showed that the absorption of conjugated dienes can be detected in the mitochondrial but not in the microsomal lipids of ethanol-treated rats. On the other hand, Corongiu et al. [35] demonstrated the absorption of conjugated dienes in microsomal lipids of ethanol-treated animals by the second-derivative spectroscopy.

An approach to the problem with different technical procedures was then devised. Since the end result of lipoperoxidation is a decrease in the most highly unsaturated fatty acids, which are the major peroxidizable substrates, a decrease in their content in the lipids of isolated subcellular fractions could indicate, among other possibilities, that a peroxidative breakdown of these moieties had actually occurred in vivo. As a matter of fact, a progressive decrease in the arachidonic acid content of liver microsomal phospholipids was observed [33] shortly after carbon tetrachloride intoxication; it was also observed, in contrast with liver phospholipids, that hepatic triglycerides do not show any change in arachidonic acid content after poisoning, again suggesting that lipid peroxidation involves structural lipids rather than the lipids accumulating in the liver as a result of the intoxication. A clear decrease in the arachidonic and docosahexaenoic acid content of liver mitochondrial lipids from acutely ethanol-treated rats was actually found [34]. In contrast with the mitochondrial changes, ethanol did not induce a decrease in the most unsaturated components of the fatty acid pattern of liver microsomal phospholipids [34].

A decrease in arachidonic as well as an increase in linoleic acid content of liver mitochondrial lipids was also observed by French et al. [43] after chronic ethanol administration, but these changes were mainly attributed to alterations in the activity of the chain elongation desaturation system.

Implication of oxidative stress in ethanol toxicity would imply that either ethanol is converted, during its metabolism, to a free radical intermediate or that ethanol or its metabolites react with some nucleophile in an antioxidant molecule, thus blocking the molecule and decreasing the antioxidant potential. The latter possibility has been shown above (reaction of acetaldehyde with –SH groups of cysteine or GSH), but the loss of GSH is by far smaller than that occurring with many other GSH depletors (bromobenzene, allyl alcohol, etc. [28]) and cannot account by itself for the induction of lipid peroxidation. The former possibility—the formation of a free radical during ethanol metabolism—was postulated by Slater [113] since many years ago. Ethanol may enter free radical reaction relatively easily [111], through the interaction with some endogenous radical; the latter could give rise to a homolytic cleavage of ethanol yielding a reducing ethoxy radical (CH_3–$CH_2O^{\cdot}$), which in the presence of some oxidant would be converted to acetaldehyde:

$$R^{\cdot} + C_2H_5OH \rightarrow RH + C_2H_5O^{\cdot}$$

$$C_2H_5O^{\cdot} + X \rightarrow C_2H_4O + XH^{\cdot}$$

(from [113]).

Several endogenous radicals are known to be involved in the NADPH-cytochrome P_{450} chain; ethanol may interact at this site during its metabolism in MEOS. Also, in the scheme proposed for the action of catalase-free radical intermediates of the hydrogen donor are formed; if ethanol is the donor, free radical intermediates from ethanol can result.

More recent studies have conclusively shown that ethoxy radical is really generated during ethanol oxidation and that an oxidative stress is imposed on the liver cell as a result of ethanol metabolism [94]. Several sources of such an oxidative stress have been described. Ethanol oxidation results in the production of free radicals, which can derive from both oxygen and ethanol itself. Oxygen radicals can originate as follows: microsomal NADPH-cytochrome *c* reductase and cytochrome P_{450} (components of MEOS) can generate $O_2^{\cdot-}$ and H_2O_2 [52, 53, 65, 93, 124]; the same oxygen species can be produced by aldehydes oxidase and xanthine oxidase [85], both involved in the metabolism of ethanol-derived acetaldehyde; $O_2^{\cdot-}$ and H_2O_2 can also be generated by microsomal NADPH oxidase, which has been shown to be increased after acute [120] or chronic [70, 104, 119, 122] ethanol administration; during NADPH oxidation liver microsomes produce significant amount of $OH^{\cdot}$ (being H_2O_2 the precursor), which in turn appears to be required for ethanol oxidation [20, 26, 55].

With regard to ethanol-derived radicals, it has been shown [4, 5] that ethanol is activated to a free radical intermediate by the ethanol inducible form of cytochrome P_{450}, i.e., the specific isoenzymatic form involved in MEOS, CYP2E1. With the use of electron spin resonance (ESR) spectroscopy in combination with the spin trapping agent 4-pyridyl-1-oxo-*t*-butyl nitrone (4-POBN), it has been demonstrated [4, 5] that rat liver microsomes incubated with ethanol and NADPH can produce a free radical intermediate, identified as 1-hydroxyethyl radical. Free radical intermediates are also produced by liver microsomes during the metabolism of various aliphatic alcohols (1-propanol,

2-propanol, 1-butanol, 2-butanol and 1-pentanol), indicating the existence of a common activating pathway for these compounds [5, 7]. The formation of radical intermediates has been confirmed in the whole animal in vivo with the use of 4-POBN [8, 60, 102, 103]. The generation of ethanol radicals would occur during the process of univalent reduction of dioxygen and possibly would be carried out by ferric cytochrome P_{450} oxy-complex (P_{450}–$Fe^{3+}O_2^{\cdot-}$) [10, 11] formed during the reduction of heme-oxygen. In such a state, cytochrome would be sufficiently reactive to abstract a proton from the 1-carbon of ethanol, yielding a carbon-centered radical and H_2O_2 [116]. Alternatively, hydroxyethyl radicals could be produced by addition to ethanol of $OH^{\cdot}$ radicals generated by liver microsomes [81]. However, generated hydroxyethyl radicals bind to microsomal protein [9], particularly CYP2E1, and probably play an important role in the induction of lipid peroxidation [6, 42]. The binding of alcohol radical to protein represents another mechanism of hepatic protein alkylation in addition to that operated by acetaldehyde [41] and known to contribute to overall liver cell damage. Furthermore, the hydroxyethyl radical-derived protein adducts are immunogenic and give rise to antibodies different from those generated by acetaldehyde-derived protein adducts [88]. Chronic alcohol feeding of rats leads to the production of antibodies that recognize hydroxyethyl rat serum albumin but do not recognize rat serum albumin [88]. Moreover, sera of alcoholic cirrhotic patients contain IgG and IgA antibodies that recognize proteins modified by hydroxyethyl radicals [27]. Such antibodies may play an important role in the immunologic reactions triggered by ethanol and due to antibodies against liver cells found in the serum of patients with alcoholic liver injury [46].

Ethanol-induced oxidative stress as measured by F_2-isoprostane determination

A great advance in the study of oxidative stress has been represented by the demonstration [89, 90, 106] of the formation of a series of prostaglandin F_2-like compounds, named F_2-isoprostanes, which originate in vitro and in vivo from the peroxidation of phospholipids bound arachidonic acid. Since F_2-isoprostanes, which are initially formed in situ on phospholipids [91], are released into the blood compartment and since these prostanoids are much less reactive than other lipid peroxidation products such as lipoperoxides and aldehydes, they can be found more easily in plasma and urine. Elevated levels of F_2-isoprostanes have been found in various human pathologies [105]. Therefore, we reconsidered the whole problem of ethanol-induced oxidative stress with this methodological approach, which is nowadays considered as the most sophisticated and reliable technique to evaluate oxidative stress, at least when the determinations are carried out by gas-chromatography mass spectrometry. Plasma F_2-isoprostanes are increased in ethanol toxicity ([92, 86, 13, 96], Comporti et al. unpublished work).

Final considerations

Owing to the fact that this review is concerned with the mechanisms inducing oxidative stress upon ethanol exposure (particularly acute exposure), no attention has been paid to the development of alcoholic liver disease and its progression to liver fibrosis. Thus, no mention has been done of the impairment of endogenous antioxidant defences, accumulation of unfolded proteins and endoplasmic reticulum stress [84, 112], AMP-dependent protein Kinase (AMPK) and adiponectin-regulated hepatic lipid metabolism [107], translocation of gut derived endotoxins to portal circulation [98], activation of Kupffer cells and release of proinflammatory cytokines (TNFα in particular), role of immune reactions in alcoholic-induced inflammation and progression to liver fibrosis through activation of hepatic stellate cells.

Extensive information about the above topics can be found in several reviews [2, 3, 79].

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